



ORYZA OIL & FAT CHEMICAL CO., LTD.



EU-ORGANIC  
CERTIFICATE



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**ECOCERT**  
RAW MATERIAL  
**COSMOS**  
APPROVED



*Deodorant Effect of Body-odor*  
**cosmeHerbest™ BLACK CUMIN**  
**Nigella Sativa Seed Extract**



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## 1. Introduction

It is said that Japanese people have comparatively little body odor. The reasons for this are considered to include diet, body type, climate, and the practice of daily bathing. As a result, it is even more noticeable when people with pungent body odor appear nearby. It is possible that our own body odor is disturbing those around us because it is difficult for us to realize our own body odor and those around us hesitate to point it out.

Our bodily smells, that can be summarized in the phrase “body odor”, are generated from a variety of different areas, and depending on the bodily location, the “type of odor” is also different, and the “type of odor” and “area that generate the odor” also differ depending on the age. The sources of the odors are mainly sweat and sebum secreted from the body, and normal inhabitants in the skin.

The skin contains two types of sweat (eccrine sweat and apocrine sweat) and two types of sebum (sebum generated by the sebaceous glands and sebum in the epidermis), which are “substances that cause an odor”. In fact, sweat and sebum do not smell directly after being secreted. The sweat secreted by the eccrine glands found in the entire human body is called eccrine sweat. It mainly consists of water but also includes slight amounts of sodium chloride, lactic acid, and urea etc. The apocrine glands, on the other hand, are the sweat glands existing with hair, and are only present in specific areas, such as the armpits, areola, and pubic area. As the apocrine sweat excreted from this area contains protein, fat, fatty acids, cholesterol, glucose, ammonia, and iron, it is cloudy and generates an odor. It is said that this originally played the role of pheromones. As time passes, sweat mixes with sebum and they are metabolized and broken down by bacteria and also oxidized with oxygen in the air, generating odorous substances.<sup>1)</sup>

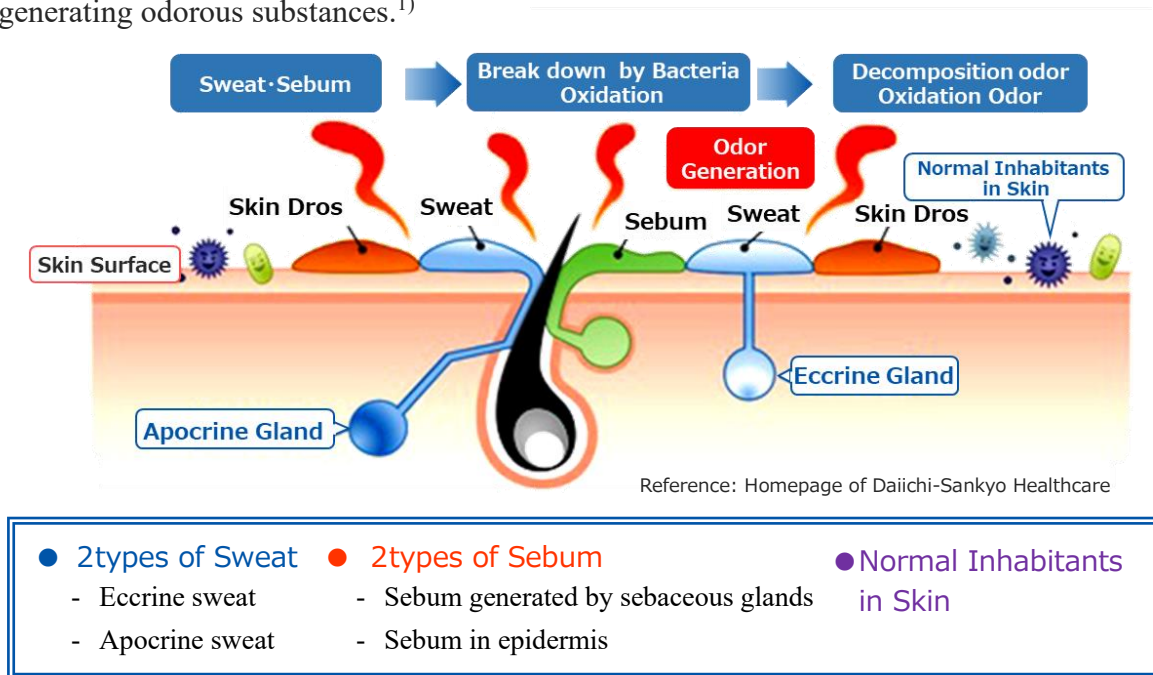


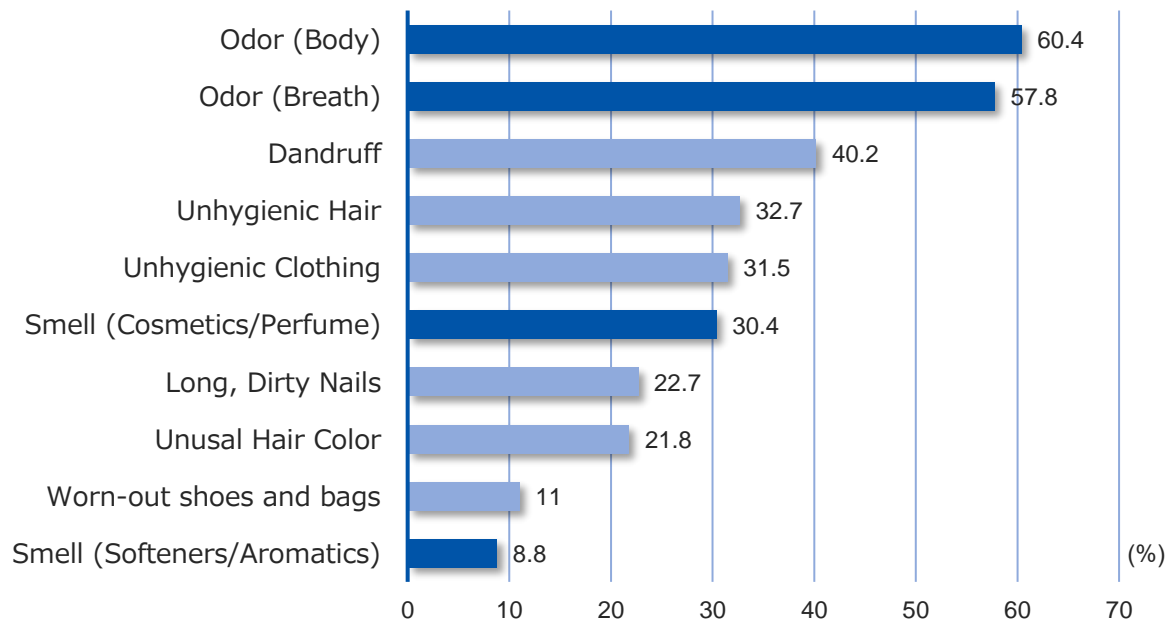
Fig. 1: Sweat Glands / Apocrine glands and Eccrine glands

## 2. Smell Harassment<sup>2)</sup>

Since around 2013, the term “smell harassment” has been used, and recently this has been abbreviated to “Sume-hara” in Japanese. This term refers to making those around you feel uncomfortable with your body odor etc. Today, this is a Japanese-English word used in everyday conversation. Whereas this is a type of “harassment”, it includes not only intentional situations, but also often includes cases where the person concerned inconveniences those around them without realizing it. This is becoming a social issue in Japan, with cases appearing in which smell harassment is used as a reason for dismissal from employment. The body odor of others, despite being the thing we most want them to deal with, is the most difficult thing to point out, and as something even difficult for family members to mention, alluding to it is even more difficult in the workplace from a management point of view. People should understand the causes of body odor and use skin care and hair care products that can reduce it before causing distress to others without realizing it. For this reason, it is believed that demand for such products will increase even more.

### Q1 Things that bother people for workplace appearance and grooming

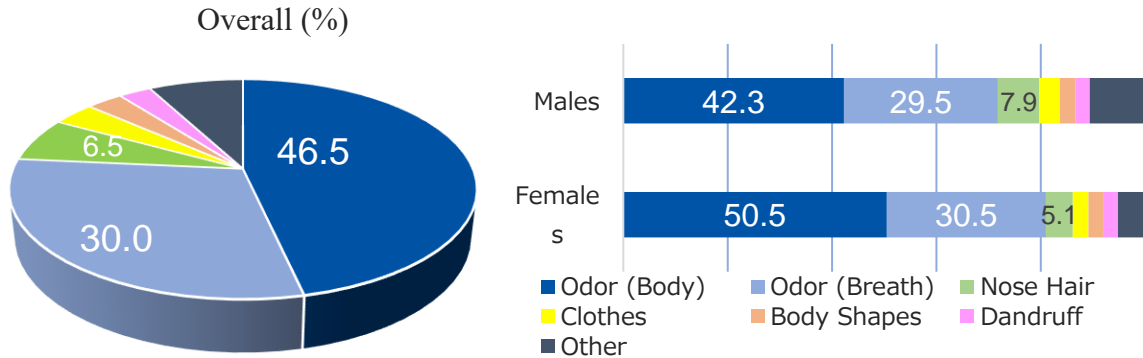
What kind of things bother you in terms of the appearance and grooming of those around you, such as colleagues, in the business environment (workplace)?



Source: Extracted from the General Odor Research on Male Bodily Odors (Mandom mail magazines) Workplace Version Vol. 02  
 Survey month: May 2014 / Number of surveyed persons: Working men and women 1117 people in total / Surveyed by: Mandom Corporation

## Q2 Things difficult to point out in relation to the grooming of others in the workplace

What is the thing that is most difficult to point out in others in relation to the appearance and grooming of others in business environment (workplace)?



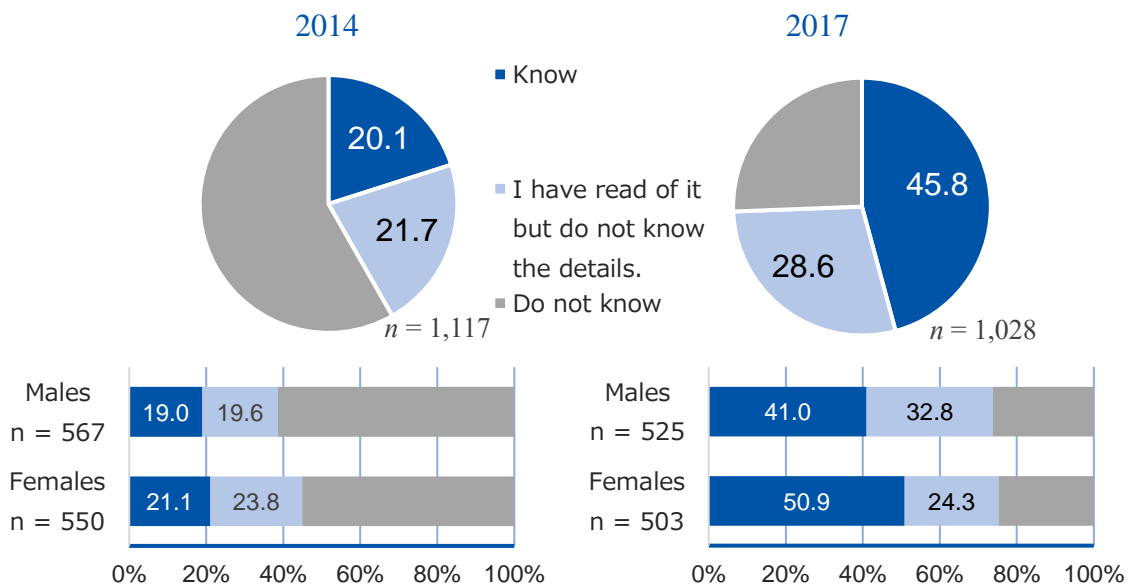
Source: Extracted from the General Odor Research on Male Bodily Odors (Mandom mail magazines) Workplace Version Vol. 02  
 Survey month: May 2014 / Number of surveyed persons: Working men and women 1117 people in total / Surveyed by: Mandom Corporation

Despite the fact that “odor (body odor)” is the thing people are bothered the most, it has also been revealed to be the thing most “difficult to point out”. It seems that those around us do not inform us of our own body odor. Unless we care for ourselves, we may cause trouble for other people around us without realizing it?!

When comparing men and women, women feel more strongly that it is “difficult to point out” odors. It is conjectured that there is the irritating situation that despite the fact that they are more sensitive to odor than men, they find it more difficult to allude to the odors.

## Q3 Smell Harassment (Sume-hara)

Do you know the word “Smell Harassment” (Sume-hara) ?

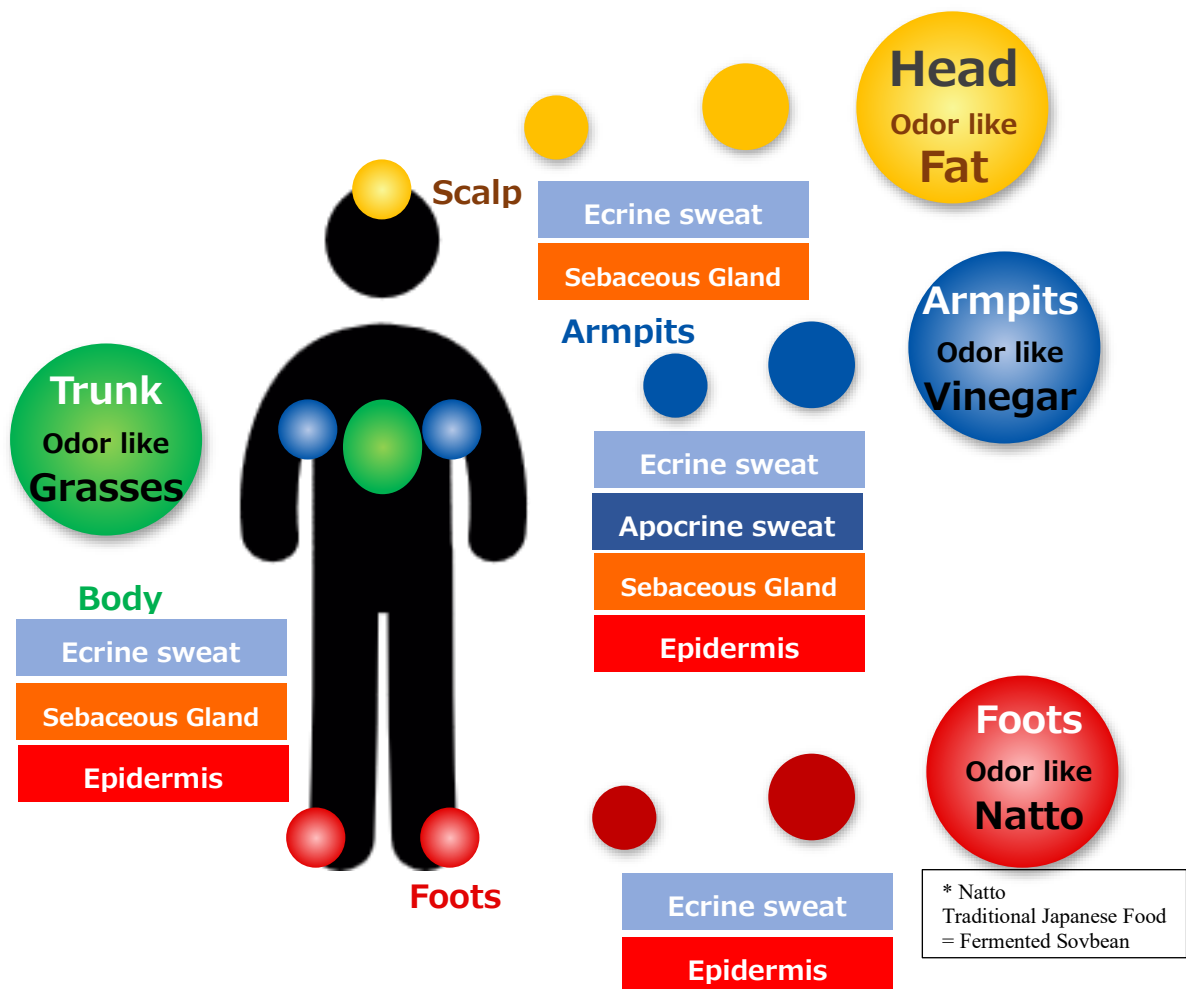


**The awareness rate of “smell Harassment” has more than doubled to 45.8% in the last 3 years.**

Source: Extracted from the General Odor Research on Male Bodily Odors (Mandom mail magazines) Workplace Version Vol. 05  
 Survey month: May 2014 / Number of surveyed persons: Working men and women 1117 people in total / Surveyed by: Mandom Corporation  
 Survey month: May 2017 / Number of surveyed persons: Working men and women 1028 people in total / Surveyed by: Mandom Corporation

### 3. “Type of Body Odor” and “Substances that Cause an Odor”

The type of odor differs depending on the body part. This occurs because the “substances that cause an odor” differs depending on the body part. “Substances that cause an odor” are two types of sweat (eccrine sweat and apocrine sweat) and two types of sebum (sebum secreted from sebaceous glands and sebum in the horny layer). Different odorous substances are generated according to the combination of these substances. Additionally, there are a wide variety of bacteria attached to the skin (normal inhabitants in the skin). Normal inhabitants in the skin are present in the moisture barrier which is formed by sebum and moisture mixed in a fine balance. Sebum and sweat are almost odorless when they are just secreted. However, as time passes, through the working of the normal inhabitants in the skin, contained components such as fats, proteins, and amino acids oxidize and are broken down, producing an unpleasant-smelling gas (volatile components). So far, several hundred varieties of body odor components have been confirmed.



Source: Extracted from the General Odor Research on Male Bodily Odors (Mandom mail magazines)

Reference: Basic knowledge of smell, Mechanism of male body odor and coping method

Fig. 2: Type of Body Odor and Substances that causes an Odor



#### 4. Body Odor Changes due to Aging

As young people have a lively metabolism and sweat a lot, they produce so-called “sweat odor (underarm odor)”, and after reaching its peak in their 20s, this starts to gradually decrease. Then, after passing mid-30s, many people start to feel that their body odor has changed, which can be recognized by other people as well. Body odor really changes with age. Formerly, all body odors after mid-30s were referred to as “aging odor”. However, in November 2013, it was revealed, through a study by Mandom Corporation, that the odor generated around one’s mid-30s is a third odor called “middle-age greasy odor” which is different from aging odor. “Middle-age greasy odor” reaches its peak in one’s 40s and then gradually reduces in intensity. Then from around the mid-50s, “aging odor” occurs, and the generation of “sweat odor (underarm odor)” and “middle-age greasy odor” decreases. We shall pay particular attention to the odor of middle-aged men from mid-30s to mid-50s. Men in this generation have the most body odor elements in their lifetimes because the three types of odors “sweat odor (underarm odor)”, “middle-age greasy odor”, and “aging odor” are all present.

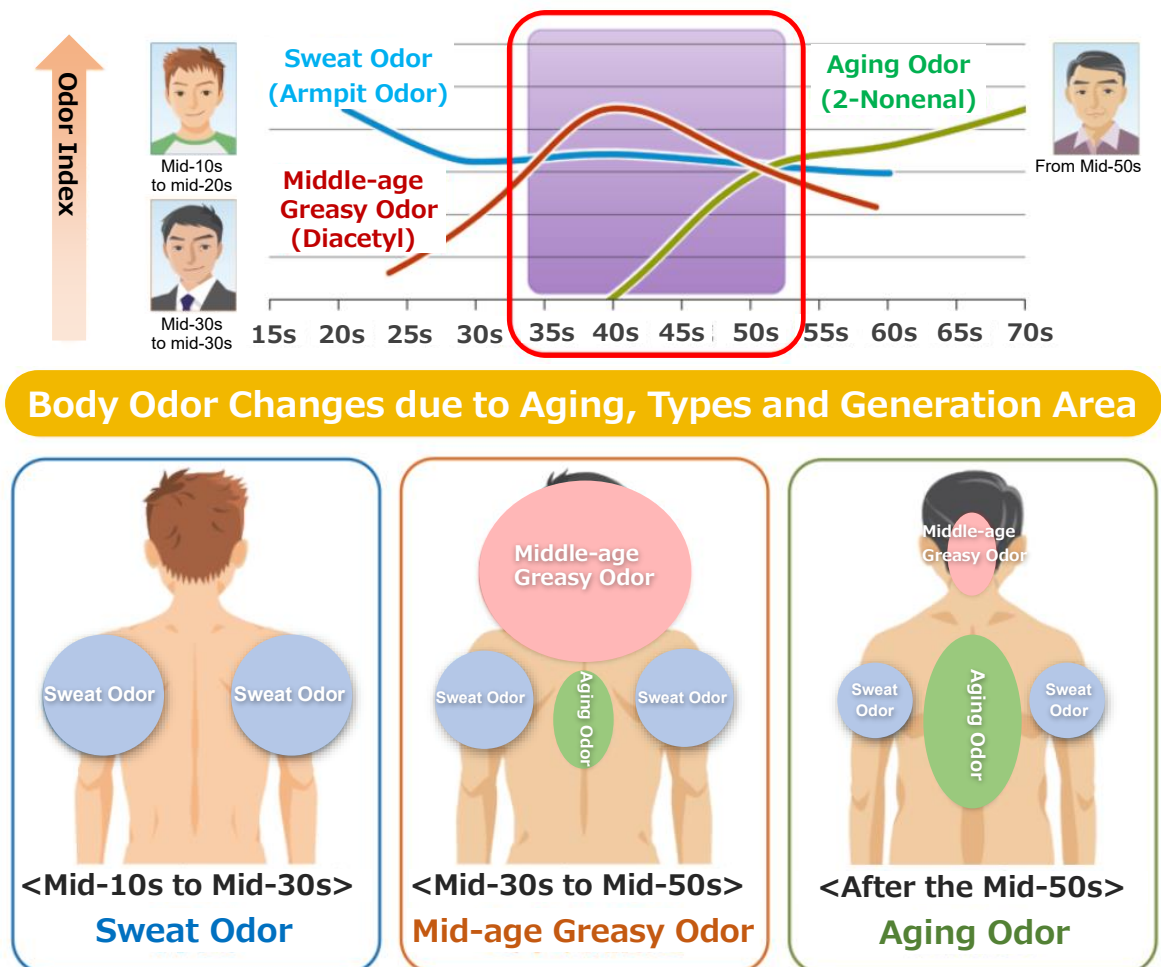


Fig.3 : Body-odor Changes due to Aging

Source: Extracted from the General Odor Research on Male Bodily Odors (Mandom mail magazines)

Reference: Basic knowledge of smell, Mechanism of male body odor and coping method

## 5. Normal inhabitants in the Skin and Body Odor<sup>3)</sup>

The sebaceous glands secreting sebum are distributed across almost the entire body (only not present on the palms of hands and bottom of the feet) and the number differs depending on the body part. When the parts are put in order of the number of sebaceous glands, generally, scalp → face (mainly around forehead, nose, and areas next to the nose) → chest → back → arms and legs. There are some individual differences. It is said that sebum is secreted at an average of 1 to 2 g per day and the secreted quantity tends to be higher for males than females. Additionally, sebaceous glands are more active during summer than winter. Looking by age, secretion is most active between the late-teens and 20s, with this gradually decreasing for women from the start of their 30s and for men from the start of their 50s. Sebum consists of 43% neutral lipids (triglycerides), 25% wax esters, 16% free fatty acids, 12% squalene, and 1 to 2% cholesterol and cholesterol esters.

The sebum secreted from sebaceous glands is an emulsion-like fluid containing fats and other substances, and 1/4 of this is secreted in the form of glycerin-fatty acid esters. Glycerin-fatty acid esters are broken down into fatty acids and glycerin by *Staphylococcus epidermidis*, which is a normal inhabitant in the skin, maintaining the skin surface acidic. Therefore, *Staphylococcus epidermidis* is often introduced as “moisture bacteria” or “good bacteria”. However, mid-lower fatty acids, such as diacetyl, pelargonic acid, isovaleric acid, butyric acid, and acetic acid are produced when glycerin-fatty acid esters are broken down and fatty acids are produced. These mid-lower fatty acids cause body odors.

Additionally, the “bad bacteria” *Staphylococcus aureus*, which is in the same *Staphylococcus* family as *Staphylococcus epidermidis* is also resident as a normal inhabitant in the skin. To maintain the skin flora and reduce body odor, it is vital to temporarily suppress the activity of these bacteria and restrict the generation of body odor substances. However, strong synthetic antibacterial agents and disinfectants cause a reduction in the “good” *Staphylococcus epidermidis*, and increase the “bad” *Staphylococcus aureus*, destroying the skin flora balance which leads to skin problems. Therefore, there is a demand for natural ingredients with mild antibacterial action using plant extracts or their components that can suppress body odor by temporarily reducing the total number of normal inhabitants in the skin without upsetting the skin flora balance.

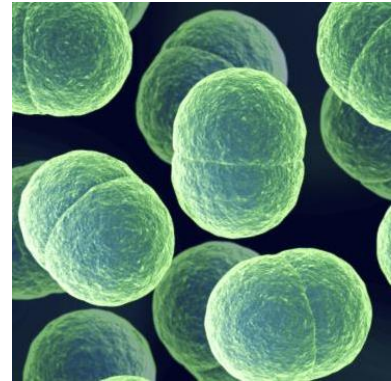
Table 1: Diacetyl and Acetoin Formation of *Staphylococcus* and *Cornebacterium*<sup>4)</sup>

| Bacterial Strains                            | Diacetyl (μM) | Acetoin (μM)   |
|--|---------------|----------------|
| <i>Staphylococcus aureus</i> NBRC13267       | 7.82 ± 0.30   | 261.32 ± 24.31 |
| <i>Staphylococcus epidermidis</i> IAM1296    | 2.67 ± 0.09   | 127.85 ± 5.16  |
| <i>Staphylococcus hominis</i> ATCC35982      | 0.20 ± 0.04   | 23.49 ± 10.4   |
| <i>Staphylococcus coptis</i> ATCC2784        | < 0.12        | < 17.00        |
| <i>Staphylococcus haemolyticus</i> ATCC29970 | < 0.12        | < 17.00        |
| <i>Cornebacterium striatum</i> ATCC694       | < 0.12        | < 17.00        |
| <i>Cornebacterium jeikeium</i> ATCC43734     | < 0.12        | < 17.00        |
| <i>Cornebacterium xerosis</i> NBRC12684      | < 0.12        | < 17.00        |
| <i>Cornebacterium minutissimum</i> ATCC12248 | < 0.12        | < 17.00        |



- *Staphylococcus epidermidis*

This is mainly resident in the nasal cavities and epidermis, and is normally non-pathogenic. The bacteria play the role of maintaining the health of the epidermis by protecting it from other pathogenic bacteria. *Staphylococcus epidermidis* and *Propionibacterium acnes* play the role of breaking down the fat in the sebum using enzyme lipase, separating the mildly acidic fatty acids. The free fatty acids act as an antibacterial barrier against other bacteria. When *Staphylococcus epidermidis* works actively, alkalophilic *Staphylococcus aureus* is unable to propagate. It can be said that, through this balance, a good state of skin flora is maintained. However, mid-lower fatty acids produced when glycerin-fatty acid esters are broken down by *Staphylococcus epidermidis* are a causal substance of body odor. Foot odor is caused by the short-chain fatty acid isovaleric acid. *Staphylococcus epidermidis* is believed to be involved with the generation of the substance.



- *Staphylococcus aureus*

*Staphylococcus aureus* is a normal inhabitant that exists in the surface of human skin and the follicles and is particularly resident in the nasal cavities. Since it has a high salt tolerance and it can proliferate under 10% food salt concentration, it can easily survive even through human sweat. *Staphylococcus aureus* is known for being toxic and it generates a toxin called enterotoxin when it proliferates within food. This toxin eaten with food causes harm to humans. *Staphylococcus aureus* itself is weak against heat. However, enterotoxin is not broken down even when it is heated at 100 °C for 30 minutes so this bacterium can cause food poisoning.



- *Corynebacterium xerosis*

Armpit odor is generated by odor components caused by substances secreted when the sebum secreted by the apocrine sweat glands and proteins are broken down by *Corynebacterium xerosis* (also referred to as armpit bacteria). Distinctive underarm odor is believed to occur when *Corynebacterium xerosis* gains prominence over *Staphylococcus epidermidis* in the skin flora.

- *Propionibacterium acnes*

*Propionibacterium acnes* is anaerobic bacteria and breaks down sebum into 3-carbon propionic acid and glycerin. This fatty acid forms a moisture barrier and protects the skin from the invasion of pathogenic bacteria and UV rays. However, when it propagates excessively, it causes suppurative inflammation of the skin (pimples). This bacteria is well known as the cause of pimples. The odor of most pimples with an offensive odor derives from propionic acid, and it is possible that areas where the pimples suppurate and turn yellow have the above-described *Staphylococcus aureus* growing.

- *Malassezia furfur*

*Malassezia furfur* is a fungus and is also known as the dandruff bacteria. In particular, most fat-based dandruff is said to be caused by this bacteria. This is the basidiomycetes system yeast resident in the skin of humans and animals. As it requires fat to propagate, it easily attaches to the scalp, which has a high fat content.

## 6. Oxidation of Sebum and Body Odor

Odor of most body odor components is generated by mid-lower fatty acids generated when glycerin-fatty acid esters, which comprise the majority of sebum, are broken down by normal inhabitants in the skin. However, Nonenal, known as “aging odor”, is caused by the oxidation of fatty acids.

Fatty acids secreted from sebum change as we grow older. From our early 40s, the amount of palmitoleic acid secreted from sebum increases. Additionally, one of the sebum components, squalene, is peroxydased by UV rays etc., and becomes squalene monohydroperoxide. When the previously described palmitoleic acid is peroxydased, 2-nonenal that causes aging odor is generated.<sup>5)</sup> Nonenal, which causes aging odor, is known to increase with the secretion of palmitoleic acid.

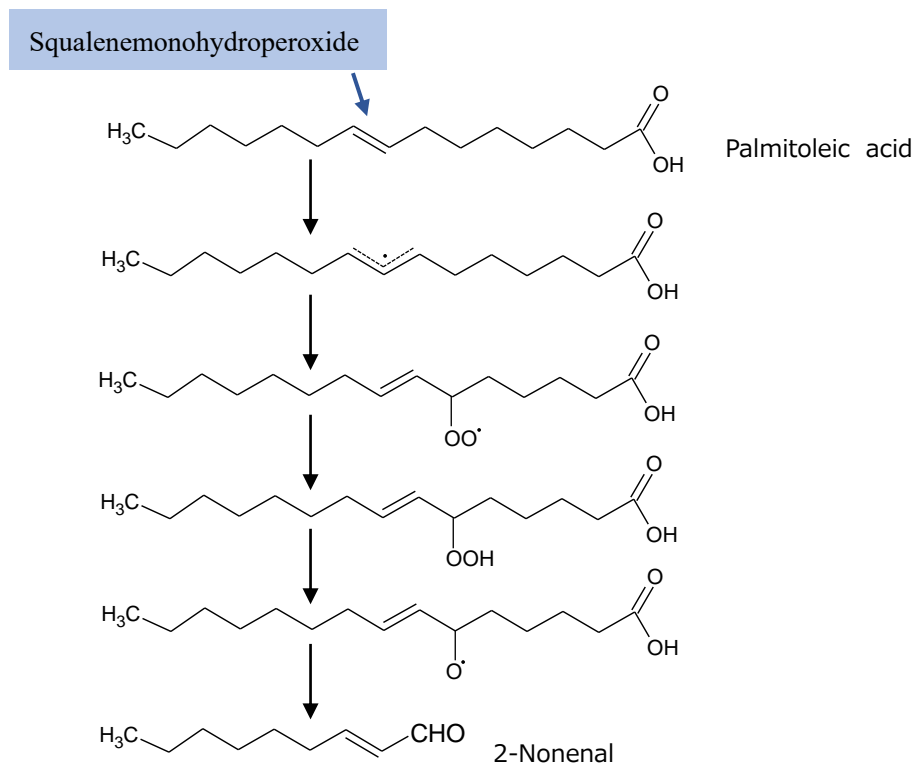


Fig. 4: Generation Mechanism of Nonenal

## 7. Summary in Regard to Body Odor

Body odor components are generated by two types of sweat that are apocrine sweat and eccrine sweat, two types of sebum that are secreted from the sebaceous glands and lipid originating from the horny layer, as well as normal inhabitants in the skin. Body odor components change as we age due to differences in sebum secreted. Most Japanese people have adopted the practice of washing their bodies in the bath or shower nearly every day so are said to be a race with comparatively low levels of body odor. However, as Japanese people have a strong aversion to causing inconvenience to other people, they are extremely sensitive to body odor care (odor care) compared to people in other countries. This tendency can be seen in antibacterial materials used for items such as ball pen grips and straps to hold onto in commuter trains. Lately, there are various attempts to mask body odor as well. For example, laundry softeners contain a large number of aromatic components.

Table. 2: Summarize Sweat odor, Middle-age Greasy Odor and Aging-odor

|                        | Sweat odor<br>(underarm, foot odor)  | Mid-age Greasy odor   | Aging odor  |
|------------------------|--|---|---|
| <b>Occurrence</b>      | Mid-10s to mid-30s   | Mid-30s to mid-50s  | Mid-50s and older   |
| <b>Area</b>            | Underarm, soles of feet, body in general (sweat glands)  | Parietal, occipital, nape of neck                             | Head, forehead, nose, behind the ears, behind the neck, neckline, back, chest |
| <b>Mechanism</b>       | Sebum is broken down by normal inhabitants in the skin, and this mixes further with sweat to generate an odor.   | Lactic acid is broken down by staphylococcus.                 | Palmitoleic acid is peroxydased by squalene monoepoxide oxidase.              |
| <b>Odor Components</b> | 1) Armpit Odor<br>- Sulfur odor:<br>3-Methyl-3-Sulfanylhexane-1-ol<br>- Spicy odor<br>3-Hydroxy-3-methylhexenoic acid<br>-Grease odor<br>3-Methyl-2-hexenopic acid<br>2) Foot odor<br>- Isovaleric acid<br>- Isovalerate aldehyde<br>- Butyric acid<br>3) Sweat Odor<br>- Vinegar odor<br>- Ammonia odor | - Diacetyl<br>- Paragonic acid<br>- Androstenone (male smell) | - Hexanal<br>- Heptanal<br>- Octanal<br>- Nonenal<br>- Decanal                |

## 8. Black Cumin

*Nigella sativa* (scientific name, “Nioi Kurotaneso” in Japanese) is a member of the *Ranunculaceae* Nigella family, and 20 varieties of Nigella are known in the world. The flowers are blue or white and are intensely beautiful. As their seeds are black, they are also called black seeds. The plant is 40 to 50 cm high. It has finely branched alternate leaves and one flower at the tip of each branch. The generic name comes from the Latin word Niger, meaning black, and the Japanese name Kurotaneso (plan with black seeds) is also given from the generic name. It is also known by the name “Black Cumin”, despite the fact that Cumin in the parsley family is a completely different plant. Additionally, it is referred to as Roman Coriander, Black Sesame, and Onion Seeds, and in English, has the romantic name of “Love in a Mist”.



It is an annual plant distributed from the Mediterranean coast to West Asia and is a somewhat unfamiliar plant in Japan. However, the earliest records of cultivating and using this plant stretch back to Ancient Egypt. Black cumin oil was also discovered in the burial chamber of King Tutankhamen of Egypt, which dates it as approximately 3,300 years old. In Arabic cultures, it has the meaning of “blessing seeds” as Habbatul barakah, and the Islamic prophet Mohammed talked of this seed as the “medicine to cure all diseases other than death”. The seed is used as an everyday herb and medicine in the Middle-East and Arabic region.<sup>6)</sup>

When the seeds are pulverized, they give off an aroma like nutmeg. They are used as a substitute for black pepper and sprinkled over bread and cakes. They are often used in Indian food to provide flavor or aroma. In India, they are commonly used as a digestive, insecticide, diuretic, emmenagogue, and dermatological medicine.

When developing cosmeHerbest™ BLACK CUMIN, it was found that the Black Cumin seeds contain thymoquinone belonging to the quinone family. As a result of investigating how to extract the highest amount of thymoquinone possible, a supercritical fluid extraction method was adopted, allowing us to successfully develop black cumin extract containing a thymoquinone quantity of 2% or more within the product.

## 9. Components in cosmeHerbest™ BLACK CUMIN

To produce cosmeHerbest™ BLACK CUMIN, ingredients with a low polarity are extracted mainly through a supercritical extraction method using carbon dioxide. It contains a type of quinone called thymoquinone as the main component, thymohydroquinone, as well as *p*-cymene (cymene) and alpha-thujene (thujene) with a mono terpene skeleton that is an essential oil component. This fat-soluble extract also contains saturated fatty acids such as palmitic acid and unsaturated fatty acids such as linoleic acid and oleic acid. Some literature introduces nigellone, which is a dimer of thymoquinone, as a compound isolated from black cumin. According to a report, it has been confirmed that nigellone inhibits histamine release from mast cells and the synthesis of 5-lipoxygenase.<sup>7)</sup>

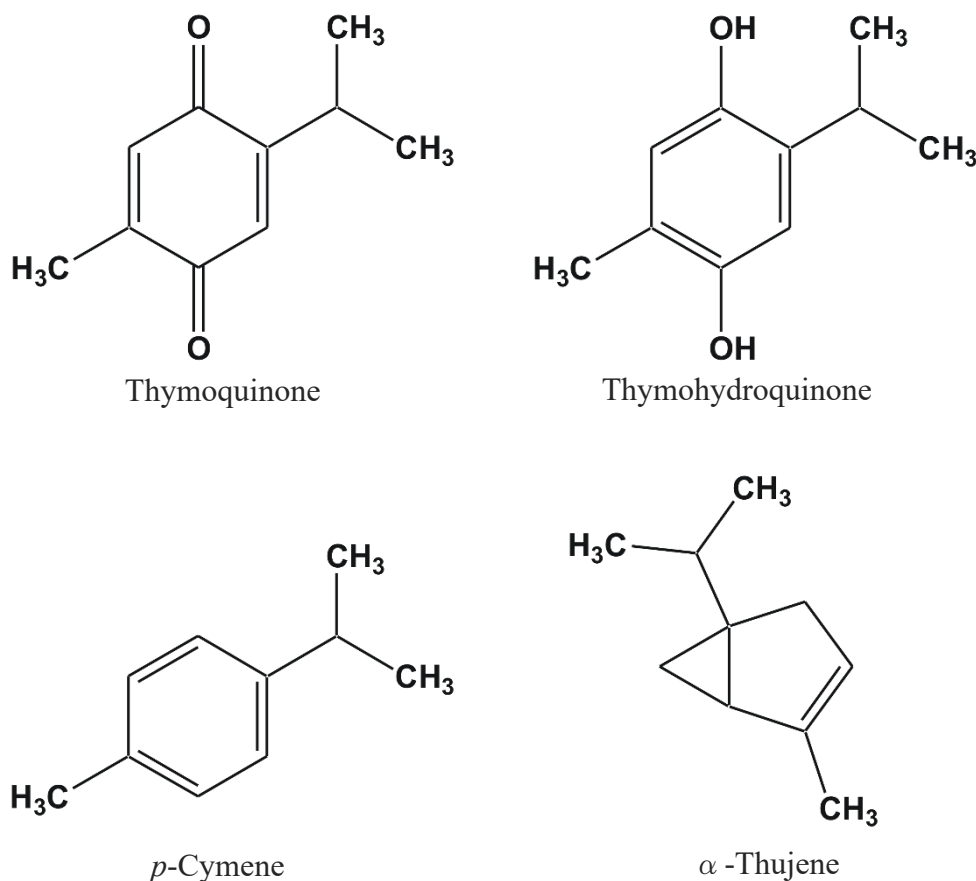


Fig. 5: Components in CosmeHerbest™ BLACK CUMIN

## 10. Effectiveness Evaluation

cosmeHerbest™ BLACK CUMIN has been confirmed to act to reduce body odor. Body odor components are generated from sebum, sweat, normal inhabitants in the skin, and oxidized sebum. As a test for preventing the generation of odors, we first conducted an antibacterial test against normal inhabitants in the skin.

Then, we conducted a sensory test to check the effect to reduce the odor of 2-nonenal using cosmeHerbest™ BLACK CUMIN and plant extracts which are commercially available as cosmetic ingredients for their deodorant action in order to check if they act to reduce odors even after odor components are generated. After that, we tested an action to suppress odorous components (acetic acid, butyric acid, isovaleric acid, and diacetyl) using a gas detector tube as well as an action to reduce the odor of 2-nonenal using chromatography. As a result, cosmeHerbest™ BLACK CUMIN was confirmed to reduce odorous components.

Finally, we conducted a test in which test subjects applied the cosmetic formula in order to observe the formula's ability to suppress odor generation. While working as usual in the company, the test subjects applied a lotion containing cosmeHerbest™ BLACK CUMIN twice a day in the morning and at night for five consecutive days to see if the odorous components would be reduced.



Antibacterial Effect against Normal Inhabitants in the Skin



Sensory Test for Deodorizing 2-Nonenal



Deodorizing Test for various Body odor components using a Detector tube



Test on Thymoquinone's Deodorizing Effect on various Body odor components using a Detector tube



Deodorizing test on 2-Nonenal and quantitative determination using a HPLC



Antioxidant Effect of Thymoquinone (quoted from literature)



Human Study on the Effect of cosmeHerbest™ BLACK CUMIN to Reduce Body odor



## 10-1 Antibacterial Effect against Normal Inhabitants in the Skin

### Test Sample

The concentration of cosmeHerbest™ BLACK CUMIN was adjusted to 50% with dimethyl sulfoxide. The material was poured into the medium for measuring the susceptibility repeatedly from 1% or 0.5% in the final product concentration (5,000 µg/ml), to 0.25%, 0.125%, 0.0625%, and down to 0.00391%. Measurement was then performed using the minimum concentration at which cosmeHerbest™ BLACK CUMIN inhibited the growth of normal inhabitants in the skin as the minimum inhibitory concentration.

### Test Method

#### <Test Microorganism>

- 1) NBRC16721 / *Corynebacterium xerosis*
- 2) TIMM2782 / *Malassezia furfur*
- 3) NBRC12732 / *Staphylococcus aureus* subsp. *Aureus*
- 4) NBRC12993 / *Staphylococcus epidermidis*

#### <Test Bacterial Fluid>

- 1) NBRC16721 / *Corynebacterium xerosis*  
 Pre-fermentation: Soybean·Casein·Digest Agar Medium (Eiken Chemical), 30 ± 1 °C、2days  
 Adjustment: Soybean·Casein·Digest Agar Medium (Eiken Chemical)  
 Number of bacteria: about 10<sup>6</sup> / mL
- 2) TIMM2782 / *Malassezia furfur*  
 Pre-fermentation: Potato Dextrose Agar with 1% Olive Oil (Difco.), 25 ± 1 °C、18-20 hours  
 Adjustment: Glucose Peptone Medium with 1% Olive Oil (Nissui Pharm)  
 Number of bacteria: about 10<sup>6</sup> / mL
- 3) NBRC12732 / *Staphylococcus aureus* subsp. *aureus* and
- 4) NBRC12993 / *Staphylococcus epidermidis*  
 Pre-fermentation: Mueller Hinton Broth (Difco.), 37 ± 1 °C、18-20hours  
 Adjustment: Mueller Hinton Broth (Difco.)  
 Number of bacteria: about 10<sup>6</sup> / mL

#### < Susceptibility measurement medium and culture conditions>

- Test bacteria 1): Soybean·Casein·Digest Agar Medium (Eiken Chemical), 30 ± 1 °C、4days  
 Test bacteria 2): Sabouraud Agar Medium with 1% Olive Oil (Eiken), 25 ± 1 °C、5-7days  
 Test bacteria 3) and4): Mueller Hinton Broth (Difco.), 37 ± 1 °C、18-20 hours

<Test Operation and Judgement>

The solutions containing the bacteria to be tested were streaked for approximately 2 cm on a plate for measuring susceptibility using a plastic loop (I.D. 1 mm), they were cultivated for a certain period, and the minimum concentration at which the growth of bacteria was inhibited was considered as the minimum inhibitory concentration (MIC).

<Test Result>

Table 3: Antibacterial Effect against Normal Inhabitants in the skin on cosmeHerbest™ BLACK CUMIN

| Concentration (%)                 | Dose of CosmeHerbest™ BLACK CUMIN |      |       |       |       |       |       |       |
|-----------------------------------|-----------------------------------|------|-------|-------|-------|-------|-------|-------|
|                                   | 0.5                               | 0.25 | 0.125 | 0.063 | 0.031 | 0.016 | 0.008 | 0.004 |
| Content of Extract (µg/mL)        | 5000                              | 2500 | 1250  | 625   | 312.5 | 156.3 | 78.1  | 39.1  |
| <i>Corynebacterium xerosis</i>    | -                                 | -    | -     | -     | +     | +     | +     | +     |
| <i>Malassezia furfur</i>          | +                                 | +    | +     | +     | +     | +     | +     | +     |
| <i>Staphylococcus aureus</i>      | -                                 | -    | -     | -     | -     | +     | +     | +     |
| <i>Staphylococcus epidermidis</i> | -                                 | -    | -     | -     | -     | +     | +     | +     |

- : Growth is not admitted, + : Accept growth of Bacteria

<Consideration>

The antibacterial action of cosmeHerbest™ BLACK CUMIN against normal inhabitants in the skin was evaluated. The inhibition of growth of *Corynebacterium xerosis* was confirmed at the final product concentration of 0.063% and *Staphylococcus aureus* and *Staphylococcus epidermidis* at 0.031%. However, no effect was observed against the dandruff fungus *Malassezia furfur*.

*Staphylococcus* is deeply involved in both the smell of sweat and middle-age greasy odor. Since cosmeHerbest™ BLACK CUMIN was confirmed to have an effective antibacterial effect against *Staphylococcus aureus* and *Staphylococcus epidermidis* as compared to other tested bacteria, it is expected to prevent body odor caused by the odor of decomposition of sebum.

## 10-2 Sensory Test of Deodorizing Effect against 2-Nonenal<sup>8)</sup>

### <Preparation of Test Sample>

The test was conducted using cosmeHerbest™ BLACK CUMIN and plant extracts which are commercially available as cosmetic ingredients for their deodorant action. Uji Green Tea Extract, Persimmon Leaf Extract-GB (J), Scutellaria Baicalensis Root Extract-BG, Sugar-cane Extract-BG (all manufactured by Yamada Drug Discovery & Development Co., Ltd.) and cosmeHerbest™ BLACK CUMIN lot No. N-802 were used.

Table.4: Plant Extracts having Antibacterial Effect on Commercially Available

| No. | Test Sample                  | LOT No. | Solid (%) | Extract (mL) | EtOH (mL) |
|-----|------------------------------|---------|-----------|--------------|-----------|
| 1   | Uji-Green Tea Extract-BG     | 17B01R  | 1.55%     | 3.23mL       | 1.77mL    |
| 2   | Persimmon Leaf Extract-BG(J) | 17B02K  | 1.26%     | 3.96mL       | 1.04mL    |
| 3   | Scutellaria Root Extract-BG  | 16C23G  | 2.62%     | 1.91mL       | 3.09mL    |
| 4   | Sugar-cane Extract-BG        | 14K21S  | 1.61%     | 3.10mL       | 1.90mL    |
| 5   | CosmeHerbest™ BLACK CUMIN    | N-802   | 5.50%*    | 0.91mL       | 4.09mL    |
| 6   | 2-Nonenal・Ethanol Solution   |         |           | 100μL        | 5mL       |

· Test Sample No.6: trans-2-Nonenal: manufactured by Tokyo Kasei Industry Co., Ltd.Lot OYV5D-ER

· Test Samples No.1 to 4 are added ethanol to make 5mL in order to adjust 1% solid matter.

\* Test Sample No.5 is added ethanol to make 5mL in order to adjust 1% essential oil as active.



1. Green Tea 2. Persimmon 3. Scutellaria 4. Sugar-cane 5. Black Cumin 6. Nonenal

### <Test Method>

Dehydrated ethanol was added to adjust the concentration of solid content or essential oil components to 1% and the solutions were adjusted to 5 ml as shown in the table above. Then, 100 μL of 2-nonenal was added to each solution and the concentration of 2-nonenal was adjusted to approximately 20 ppm. Then, seven testers ranked the intensity of odor of 2-nonenal. The testers observed the intensity of the odor of 2-nonenal while sniffing grounded roasted coffee beans before a sensory test for each sample solution in order to maintain their sense of smell.

<Test Result>

Table.5: Deodorant Effect against Nonenal of cosmeHerbest™ BLACK CUMIN

| Judgement | Strong | Nonenal Odor |   |   |   | Weak (None) |
|-----------|--------|--------------|---|---|---|-------------|
|           |        | ←            |   |   | → |             |
| YK(25)♀   | 6      | 1            | 4 | 2 | 3 | <b>5</b>    |
| KI(28)♂   | 5      | 2            | 4 | 6 | 3 | 1           |
| NY(27)♀   | 6      | 1            | 2 | 4 | 3 | <b>5</b>    |
| TK(31)♂   | 6      | 2            | 3 | 4 | 1 | <b>5</b>    |
| ST(28)♂   | 6      | 2            | 1 | 4 | 3 | <b>5</b>    |
| TT(29)♂   | 6      | 2            | 3 | 4 | 1 | <b>5</b>    |
| NS(30)♂   | 6      | 3            | 4 | 1 | 2 | <b>5</b>    |

<Consideration>

As described above, the results indicated that (5) cosmeHerbest™ BLACK CUMIN reduced the odor of 2-nonenal, except for the rankings of one person. It is assumed that the distinctive smell of black cumin demonstrates a masking effect or harmonization effect so people do not smell the odor of 2-nonenal (stink bug smell).

10-3 Deodorizing Effect for various odor substances using a detector tube<sup>9)</sup>

## &lt;Test Method&gt;

The room temperature was set to between 24 to 26 °C and stabilized. Body odor components were injected into a 3 L scent bag (made by OMI ODOR-AIR SERVICE Corporation) at the specified amount and the scent bag was completely filled with air immediately after it. Then, the scent bag was closed and left for 10 minutes (scent bag A). The sample was injected into a different scent bag at the specified amount (scent bag B).

Then, scent bags A and B were connected using a silicone tube, the gas in scent bag A was completely moved into scent bag B. The bag was closed immediately and left for 10 minutes. The residual concentration of body odor substances was measured using a dedicated gas detector tube (made by Gastec Corporation). Three pieces were used for each sample. The deodorizing rate was calculated using the following formula and the average value was calculated. No sample was injected into the blank. The residual concentration of body odor substances was measured by the same procedure.

$$\begin{aligned} & \text{Body odor substance decrease rate (\%)} \text{ (BODR)} \\ & = \{(\text{blank value}) - (\text{sample value})\} / (\text{blank value}) \times 100 \end{aligned}$$

## &lt;Body Odor substances and Specified Amount&gt;

- a. Acetic acid (Fuji Film-WakoPure Chemical Corp., Special Grade, 100-fold, 10 $\mu$ L)
- b. *n*-Butyric acid (Tokyo Kasei Industry, Special Grade, 10-fold, 10 $\mu$ L)
- c. Isovaleric acid (Tokyo Kasei Industry, Special Grade, 10-fold, 10 $\mu$ L)
- d. Ammonia (Kishida Kasei, Special Grade, 10-fold, 5 $\mu$ L)
- e. Diacetyl (Tokyo Kasei Industry, undiluted as it is, 1 $\mu$ L)

## &lt;Test Sample and Specified Amount&gt;

cosmeHerbest™ BLACK CUMIN Lot No. N-802 0.5mL  
(Containing 3.4% of Thymoquinone)

## &lt;Gas Detection Tube&gt;

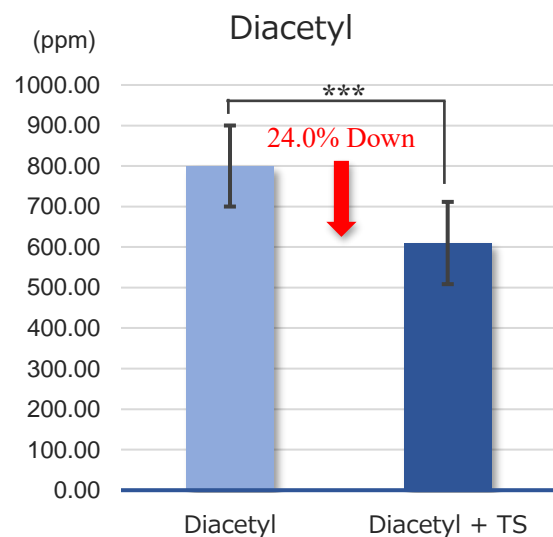
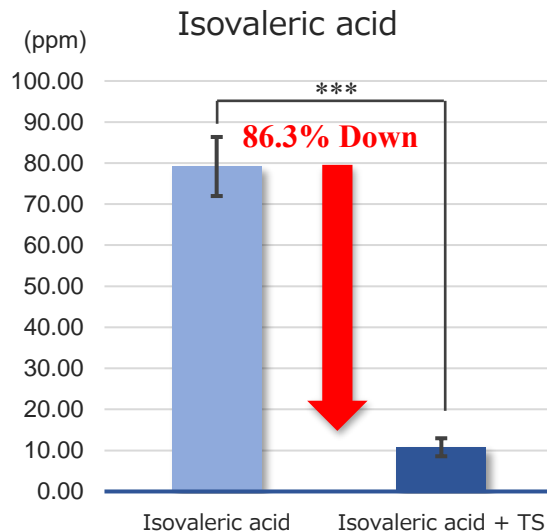
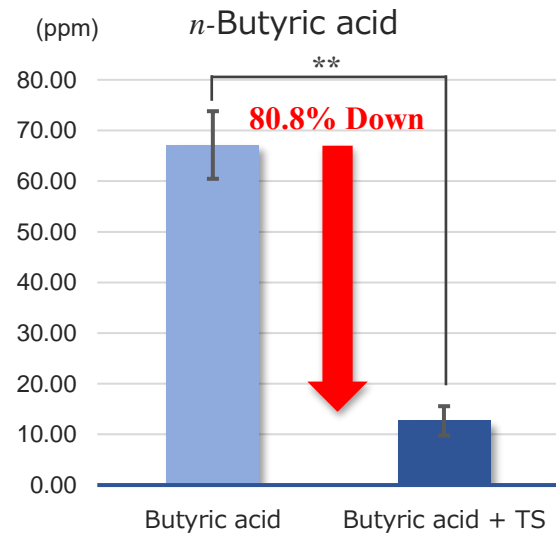
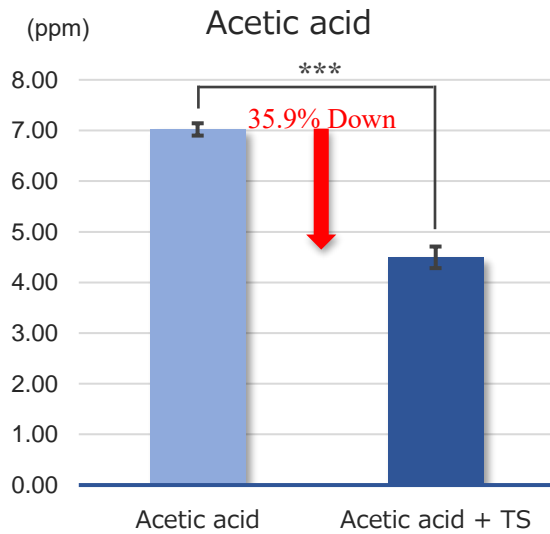
GASTEC Corporation Gas Detection Tube No. 81L / For Acetic acid  
GASTEC Corporation Gas Detection Tube No. 81 / For *n*-Butyric acid and Isovaleric acid  
GASTEC Corporation Gas Detection Tube No. 3L / For Ammonia  
GASTEC Corporation Gas Detection Tube No. 92 / For Diacetyl

<Test Result>

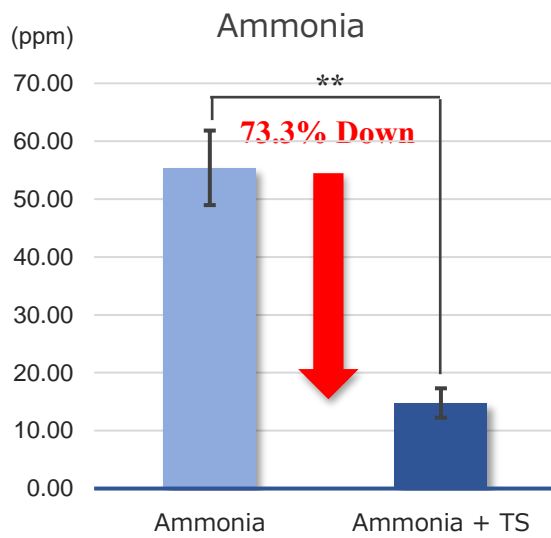
Residual concentration of various Body odor substances and Body odor substance reduction rate is as follows:

| Body odor Substances         | Determination (ppm) |       |       |         | BODR    |
|------------------------------|---------------------|-------|-------|---------|---------|
|                              | 1                   | 2     | 3     | Average |         |
| Acetic acid                  | 7.12                | 6.89  | 7.05  | 7.02    | 35.94 % |
| Acetic acid + TS*            | 4.28                | 4.51  | 4.7   | 4.50    |         |
| <i>n</i> -Butyric acid       | 74.1                | 60.8  | 66.5  | 67.13   | 80.82 % |
| <i>n</i> -Butyric acid + TS* | 9.5                 | 13.3  | 15.2  | 12.67   |         |
| Isovaleric acid              | 76.00               | 74.1  | 87.4  | 79.17   | 86.27 % |
| Isovaleric acid + TS*        | 13.30               | 9.5   | 9.5   | 10.77   |         |
| Diacetyl                     | 900                 | 800   | 700   | 800     | 24.01 % |
| Diacetyl + TS*               | 700                 | 630   | 500   | 610     |         |
| Ammonia                      | 61.75               | 55.58 | 48.88 | 55.40   | 73.30 % |
| Ammonia + TS*                | 14.82               | 17.29 | 12.22 | 14.78   |         |

\*TS = Test Sample, BODR = Body odor substance decrease rate (%)







Measurement of Ammonia Concentration by the detection tube

Significantly different from control, \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$

Fig. 6: Deodorant Effect against Body odor Substances of cosmeHerbest™ BLACK CUMIN

<Consideration>

In the test above, cosmeHerbest™ BLACK CUMIN demonstrated approximately 36% inhibition rate for acetic acid, 81% for n-butyric acid, and 86% for isovaleric acid, which was the highest. It also demonstrated a 24 % inhibition rate for diacetyl (middle-age greasy odor) and a high rate of 73 % for ammonia.

#### 10-4 Deodorizing Effect

against Body odor Substances of Thymoquinone by detection tube

In the test to deodorize body odor components, cosmeHerbest™ BLACK CUMIN was confirmed to deliver a high deodorizing performance especially on *n*-butyric acid, isovaleric acid, and ammonia.

Black cumin contains thymoquinone, which is a quinone compound. So, we studied if thymoquinone would exert a deodorizing effect.

##### <Body Odor substances and Specified Amount>

- a. *n*-Butyric acid (Tokyo Kasei Industry, Special Grade, 10-fold, 10 $\mu$ L)
- b. Isovaleric acid (Tokyo Kasei Industry, Special Grade, 10-fold, 10 $\mu$ L)
- c. Ammonia (Kishida Kasei, Special Grade, 10-fold, 5 $\mu$ L)

##### <Test Sample and Specified Amount>

Thymoquinone C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>, MW = 164.2 (made by SIGMA-ALDRICH, Lot #MKCB6982) was used. cosmeHerbest™ BLACK CUMIN used in the test described in the previous section contained 3.4% thymoquinone. Therefore, thymoquinone was resolved to 3.4% using a reagent and dehydrated ethanol so that the amount would be the same as that contained in cosmeHerbest™ BLACK CUMIN. Then, a study was carried out to check if thymoquinone can act to deodorize body odor components using 0.5 ml of the solution.

##### <Gas Detection Tube>

GASTEC Corporation Gas Detection Tube No. 81 / For *n*-Butyric acid and Isovaleric acid

GASTEC Corporation Gas Detection Tube No. 3L / For Ammonia

<Test Result>

| Body odor Substances        | Determination (ppm) |      |      |         | BO      |
|-----------------------------|---------------------|------|------|---------|---------|
|                             | 1                   | 2    | 3    | Average |         |
| <i>n</i> -Butyric acid      | 74.1                | 60.8 | 66.5 | 67.13   | —       |
| <i>n</i> -Butyric acid + TS | 9.5                 | 13.3 | 15.2 | 12.67   | 80.82 % |
| <i>n</i> -Butyric acid + TQ | 30.1                | 24.4 | 20.7 | 25.07   | 62.70 % |
| Isovaleric acid             | 76.00               | 74.1 | 87.4 | 79.17   | —       |
| Isovaleric acid + TS        | 13.30               | 9.5  | 9.5  | 10.77   | 86.27 % |
| Isovaleric acid + TQ        | 15.0                | 16.9 | 18.8 | 16.92   | 78.62 % |

- The deodorizing effect of thymoquinone against ammonia was not recognized at all.

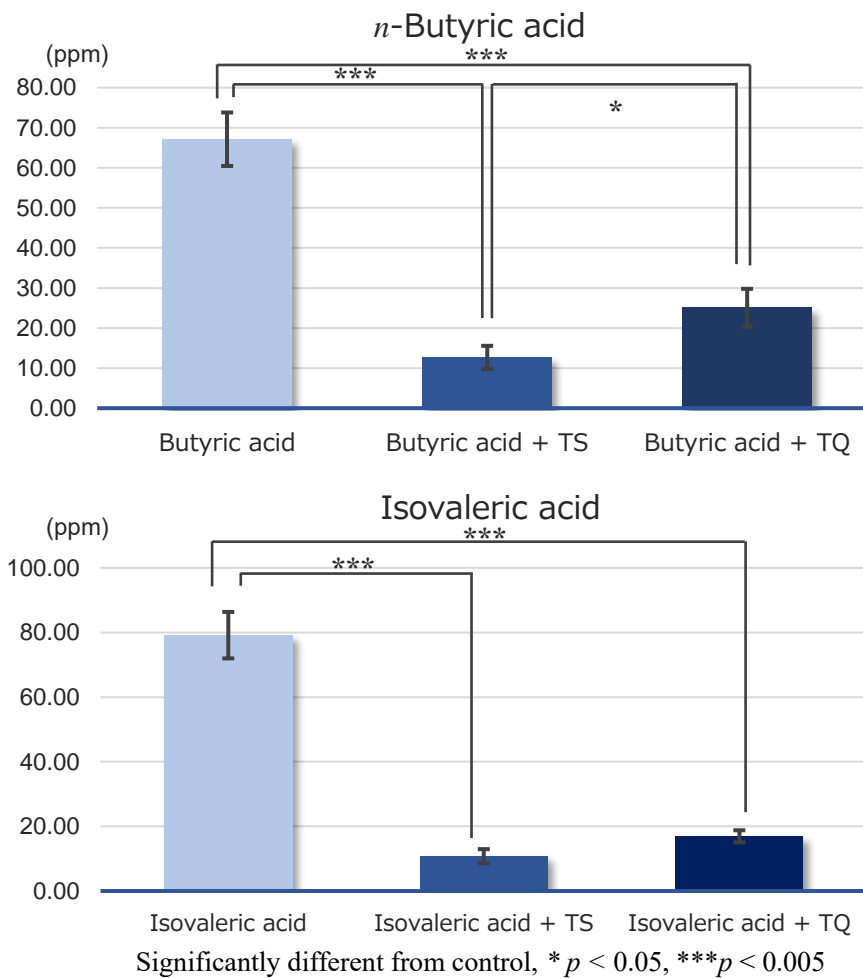


Fig.7: Deodorizing Effect of cosmeHerbest™ BLACK CUMIN and Thymoquinone

<Consideration>

It was confirmed that the action of cosmeHerbest™ BLACK CUMIN to deodorize ammonia is performed by a component other than thymoquinone. The action of cosmeHerbest™ BLACK CUMIN to deodorize *n*-butyric acid and isovaleric acid was 80.82% and 86.27% respectively. The action of thymoquinone to deodorize *n*-butyric acid and isovaleric acid was 62.70% and 78.62%, indicating that the action to deodorize *n*-butyric acid and isovaleric acid greatly depends on thymoquinone.

## 10-5 Deodorizing Effect against 2-Nonenal

In a test to study the effect to deodorize body odor components using cosmeHerbest™ BLACK CUMIN, its effect to deodorize 2-nonenal, which is an aging smell that becomes noticeable in 50s and after, was measured.

### <Test Method>

The room temperature was set to between 24 to 26 °C and stabilized. 2-nonenal and each sample were added into a 3 L scent bag (made by OMI ODOR-AIR SERVICE Corporation) at the specified amount in order to achieve the specified gas concentration. Then, the scent bag was completely filled with air. The bags were allowed to remain and 300 ml of the gas inside the bag was collected into a DNPH cartridge every 30 minutes. Then, 5 ml of acetonitrile was introduced into the DNPH cartridges containing the gas in order to induce the elution of a DNPH derivative. This eluate was measured through high-performance liquid chromatography to calculate the concentration of 2-nonenal in the bag. Analysis conditions of the high-performance liquid chromatograph and the specified amount of the sample are shown below. A blank test was also performed by the same procedure without any sample.

### <Analysis Condition by HPLC>

Apparatus : LC-20AD (Shimadzu)  
Detector : Ultraviolet absorption photometric detector  
Column : RP-Amide、Φ4.6 mm × 25 cm  
(Sigma-Aldrich Japan Corporation)  
Column Temp. : 40 °C  
Mobile Phase : Mixture of Acetonitril and Water = 80 : 20  
Flow rate : 1.5 mL/min  
Wave Length : 360 nm

### <Test Sample and Specified Amount>

cosmeHerbest™ BLACK CUMIN Lot No.N-802, 0.5 mL

### <Definition of 2-Nonenal Decrease Rate>

The 2-nonenal decrease rate was calculated using the following formula based on the measurement values obtained by the procedure described above.

$$\text{2-Nonenal Decrease Rate (\%)} = \{(\text{blank value}) - (\text{sample value})\} / (\text{blank value}) \times 100$$

The initial measurement value before adding the sample was used as the blank value and the concentration of 2-nonenal measured every 30 minutes after the test started was used as the sample value.

<Test Result>

After adding cosmeHerbest™ BLACK CUMIN, deodorizing rate of 2-Nonenal with the passage of time, is as following table:

| Test Sample                         | 使用量   | Elapsed Time (minutes) |             |             |
|-------------------------------------|-------|------------------------|-------------|-------------|
|                                     |       | 0                      | 30          | 60          |
| 2-Nonenal                           | —     | 21                     | 21 (0 %)    | 22 (-4.7 %) |
| 2-Nonenal+CosmeHerbest™ BLACK CUMIN | 0.5mL | 21                     | 10 (52.3 %) | 8 (61.9 %)  |

(Unit : ppm / The parenthesis showed the reduction rate.)

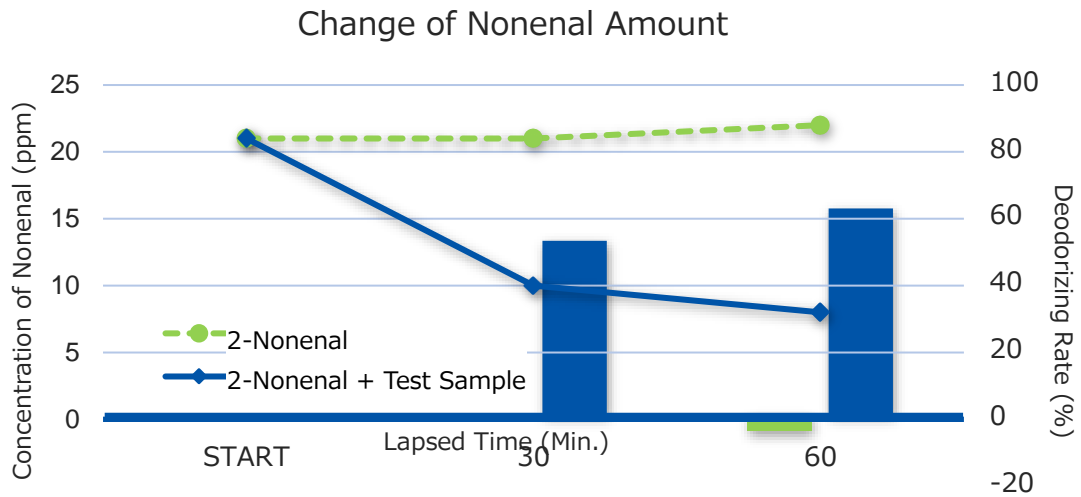


Fig. 8: Deodorizing Effect against Nonenal of cosmeHerbest™ BLACK CUMIN

<Consideration>

When compared to the blank, the sample with cosmeHerbest™ BLACK CUMIN added was confirmed to reduce 2-nonenal over time. It eliminated 52.3% of the Nonenal odor 30 minutes after adding the sample and after 60 minutes this increased to 61.9%.

According to this result, cosmeHerbest™ BLACK CUMIN is believed to have a high deodorizing effect against 2-nonenal.

### 10-6 Antioxidant Effect of Thymoquinone (quoted from Literature)<sup>10)</sup>

An *in vitro* test to study the antioxidant effect of thymoquinone has been performed by H. khither et al. at the Applicable Biochemistry Laboratory, Department of Biochemistry, University of Setif in Algeria.

According to this report, thymoquinone as an eliminator of DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals and ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salts) radicals, hydroxyl radicals (OH · ), and superoxide anion (O<sub>2</sub><sup>·-</sup>). However, it is more effective as an eliminator of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) than ascorbic acid.

| Sample        | IC <sub>50</sub> for DPPH radical (µg/mL) | IC <sub>50</sub> for ABTS radical (µg/mL) |
|---------------|---|---|
| Thymoquinone  | 125.65 ± 0.76 ***                         | 332.5 ± 14.39 ***                         |
| Ascorbic acid | 1.59 ± 0.78                               | 14.96 ± 1.68                              |

Value were presented as the mean ± SD of triplicate

\*\*\* : p ≤ 0.001

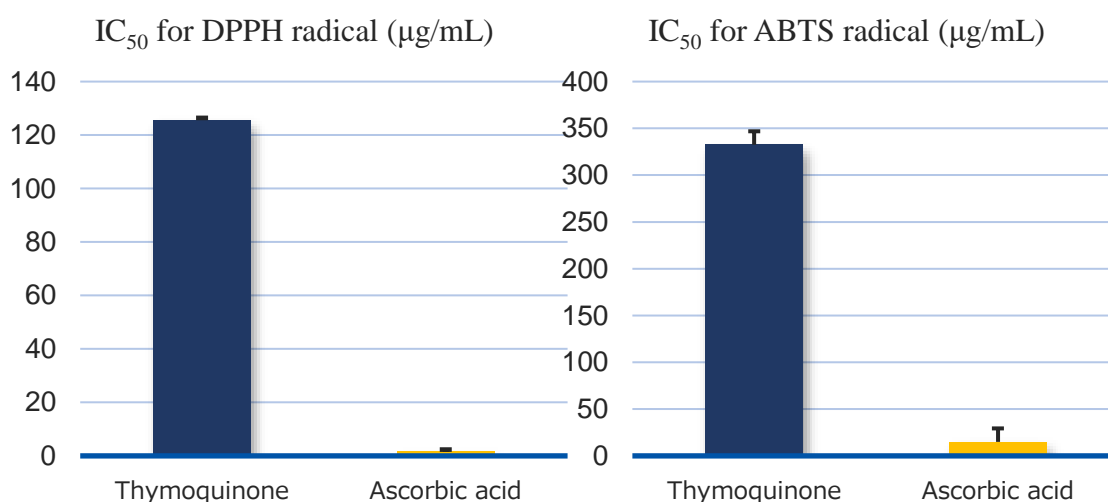


Fig. 9: Radical Scavenging Effect of DPPH and ABTS of Thymoquinone



| Sample        | IC <sub>50</sub> for OH• (µg/mL) | IC <sub>50</sub> for H <sub>2</sub> O <sub>2</sub> (µg/mL) | IC <sub>50</sub> for O <sub>2</sub> <sup>-</sup> |
|---------------|----------------------------------|--|--|
| Thymoquinone  | 26.3 ± 0.59 ***                  | 11.0 ± 0.57 **   | Less than 50%                                    |
| Ascorbic acid | 5.57 ± 0.18                      | 17.5 ± 0.56  | 54.27 ± 11.23                                    |

Value were presented as the mean ± SD of triplicate

\*\* : p ≤ 0.01, \*\*\* : p ≤ 0.001

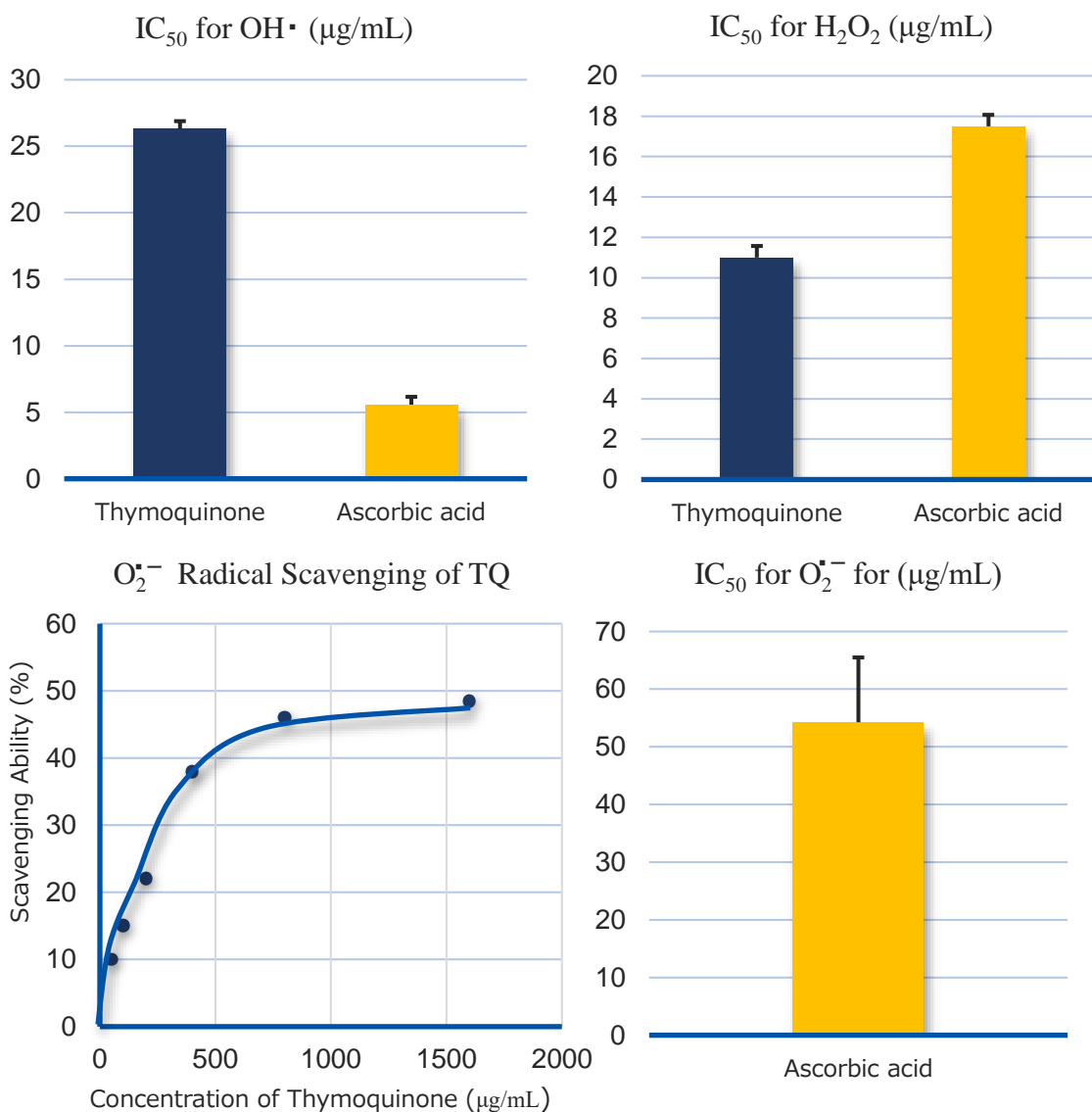


Fig.10: Elimination of various active oxygen species of thymoquinone

For super oxide anion (O<sub>2</sub><sup>-</sup>), the super oxide anion eliminating rate of thymoquinone at 1600 µg/mL was 48.55% and IC<sub>50</sub> could not be measured. Meanwhile, the super oxide anion 50% eliminating rate of ascorbic acid has been reported as 54.27 ± 11.23 µg/mL.

### 10-7 Body odor Reduction Test on Human monitors

of cosmeHerbest BLACK CUMIN<sup>10)</sup>

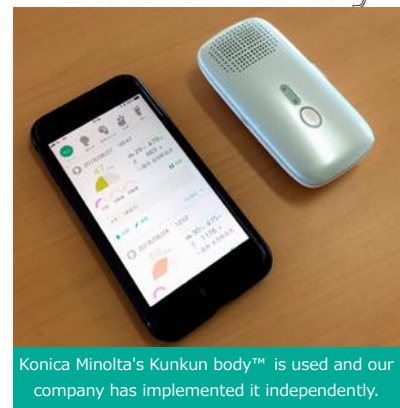
cosmeHerbest™ BLACK CUMIN has been confirmed to act to reduce nonenal odor in a sensory test and to have an effect to significantly reduce butyric acid, isovaleric acid, and ammonia in a test to study the action to deodorize body odor using a gas detector tube. In addition, cosmeHerbest™ BLACK CUMIN has been confirmed to have antibacterial properties against normal inhabitants in the skin such as *Cornebacterium* called underarm odor bacteria, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.

Based on these *in vitro* test results, we checked if the deodorizing effect occurred by applying the product on the skin.

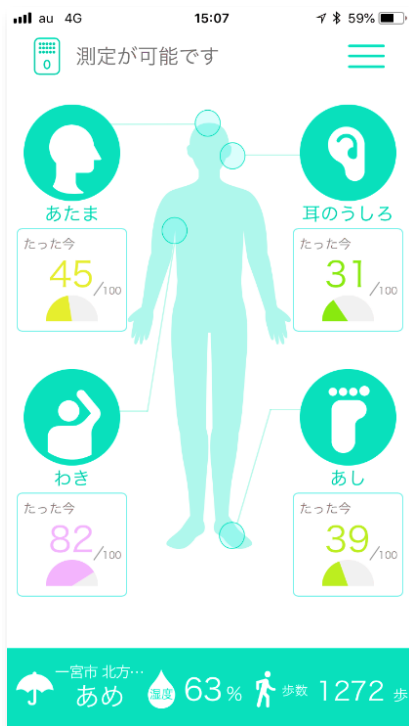
#### <Equipment Used>

Kunkun body™, is the world's first\* checker that visualizes odors in conjunction with a smartphone application. It visualizes the type and intensity of body odor. Odor is measured on the head, behind an ear, armpit, and foot and the overall body odor intensity is indicated in 100 levels. To determine the type of odor, it detects isovaleric acid and ammonia for sweat odor, diacetyl for middle-age greasy odor, and nonenal for aging odor. It indicates the odor type and intensity with bar graphs in ten levels.

kunkun body



Konica Minolta's Kunkun body™ is used and our company has implemented it independently.



#### Example of measurement data screen:

- Head odor 45/100
- Odor from behind ear 31/100
- Underarm odor 82/100
- Foot odor 39/100



Furthermore, the composition of odor types can be displayed for odors of the head and behind the ear. In this example, the odor is composed of sweat order as 5/10, and middle-age greasy odor as 5/10.

|                  |                             |
|------------------|-----------------------------|
| Sweat Odor       | : Ammonia & Isovaleric acid |
| Mid. Greasy Odor | : Diacetyl                  |
| Aging Odor       | : Nonenal                   |

\* Kunkun body™ is a registered trademark of Konica Minolta Co., Ltd.

\* Source:

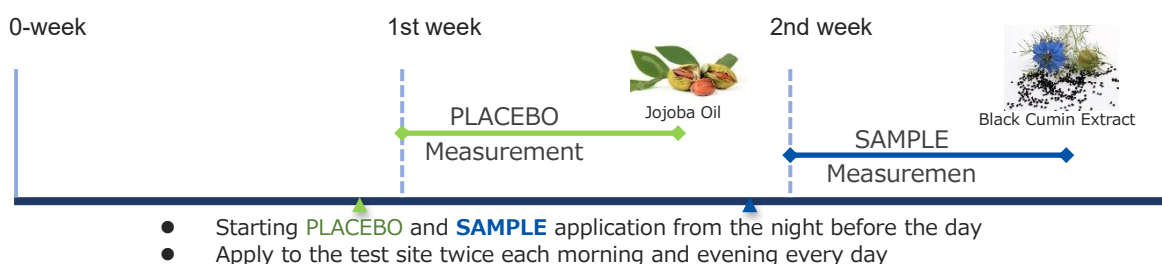
Proof and verification survey that "Kunkun body™ is "world first"  
(As of January 12, 2018) / <ESP research survey>  
(December 2017 - January 2018 survey)

### < Test Method >

Males aged from 23 to 57 (16 people in total), who thoroughly understood the purpose of the test in accordance with the Declaration of Helsinki and submitted written consent, participated in the test as subjects. The 16 people included four people each in their twenties, thirties, forties, and fifties. All of the test subjects applied the placebo lotion behind their ears, armpits, and soles twice a day in the morning and at night for five consecutive days starting from the night before the first week. The odor of the areas on which the placebo lotion was applied was measured three times a day in the morning, during the day, and at night for five consecutive days from Monday through Friday using Kunkun body™.

They then started to apply a sample lotion containing 1% cosmeHerbest™ BLACK CUMIN twice a day in the morning and at night for five consecutive days starting from the night before the second week. The odor was measured three times a day using Kunkun body™ just like the measurements for the placebo lotion.

The odor was measured in a room in which the temperature was adjusted to 25 ± 4 °C and the humidity to 60 ± 10% after being in the room for 15 minutes. The test started on August 27, 2018 and went on for two weeks until September 7.



### < Placebo and Test Sample >

Table 6: Composition of Placebo and Test Sample Lotion

| No. | Trade Name                           | Placebo | Sample | INCI Name   |
|-----|--------------------------------------|---------|--------|---|
| 1   | ELDEW PS-203 <sup>*1</sup>           | 1.50    | 1.50   | Phytosteryl/Octyldodecyl Lauroyl Glutamate        |
| 2   | EMALEX INTD-139 <sup>*2</sup>        | 1.50    | 1.50   | Isotridecyl Isononanoate                          |
| 3   | cosmeHerbest™ BLACK CUMIN            | 0.00    | 1.00   | Nigella Sativa Seed Extract,                      |
| 4   | Purified JOJOBA Oil <sup>*3</sup>    | 3.00    | 2.00   | Simmondsia Chinensis (Jojoba) Seed Oil            |
| 5   | KF-96A (6 c/s) <sup>*4</sup>         | 1.00    | 1.00   | Dimethicone                                       |
| 6   | Cetanol H <sup>5</sup>               | 3.00    | 3.00   | Cetearyl Alcohol                                  |
| 7   | EMALEX EGS-A <sup>*2</sup>           | 1.00    | 1.00   | Glycol Stearate                                   |
| 8   | EMALEX GMS-F <sup>*2</sup>           | 1.00    | 1.00   | Glyceryl Stearate                                 |
| 9   | EMALEX 8100 <sup>*2</sup>            | 1.00    | 1.00   | PEG-100 Stearate                                  |
| 10  | ORYZATOCOTRIENOL™ -90                | 0.05    | 0.05   | Tocotrienol, Tocopherol, Oryza Sativa (Rice) Bran |
| 11  | Phenoxyethanol                       | 1.00    | 1.00   | Phenoxyethanol                                    |
| 12  | EDTA-2Na (1% soln.)                  | 1.00    | 1.00   | Water, Disodium EDTA                              |
| 13  | Glycerine                            | 10.00   | 10.00  | Glycerine   |
| 14  | KeltrolCG-T <sup>5</sup> (1% soln.)  | 10.00   | 10.00  | Water, Xanthan Gum                                |
| 15  | Carbopol 940 <sup>6</sup> (1% soln.) | 10.00   | 10.00  | Water, Carbomer                                   |
| 16  | Water                                | 52.45   | 52.45  | Water   |
| 17  | Potassium Hydroxide (10% soln.)      | 2.50    | 2.50   | Water, Potassium Hydroxide                        |
|     |                                      | 100.00  | 100.00 |   |

\*1: Ajinomoto Co., Inc., \*2: Nihon Emulsion Co., Ltd., \*3: Yokozeki Oil & Fat Industries Co., Ltd., \*4: Shin-etsu Chemical Co., Ltd., \*5: CP Kelco \*6: Lubrizol Corporation

ELDEW is a registered trademark of Ajinomoto Co., Inc. EMALEX is a registered trademark of Nihon Emulsion Co., Ltd. cosmeHerbest and Oryza Tocotrienol are registered trademarks of Oryza Oil & Fat Chemical Co., Ltd. KELTROL is a registered trademark of CP Kelco. Carbopol is a registered trademark of Lubrizol Advanced Materials Incorporated.

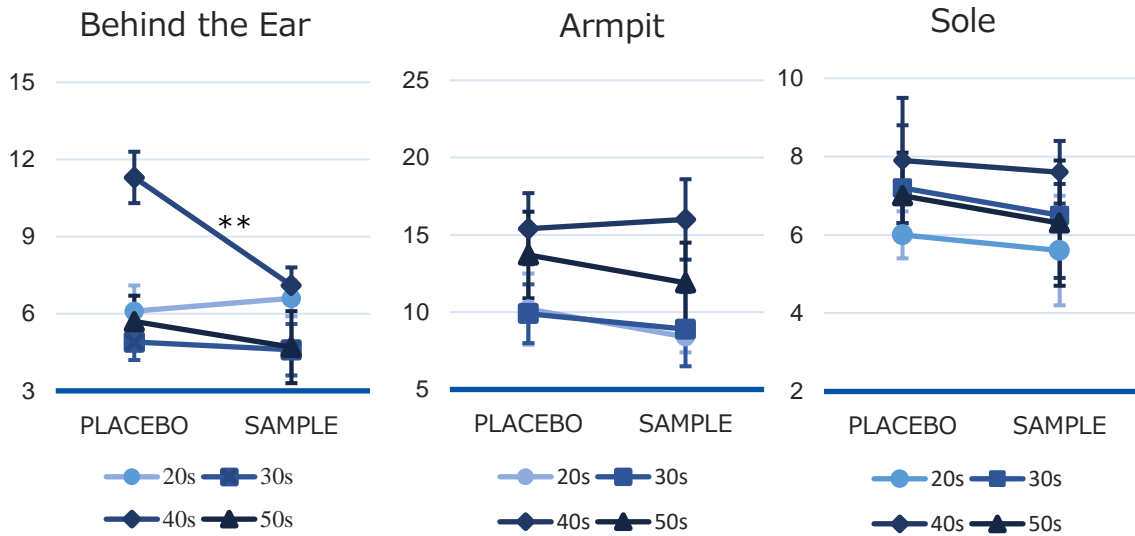
<Test Result>

The table below shows the results of measuring odors after applying the placebo lotion as well as the sample lotion containing 1% cosmeHerbest™ BLACK CUMIN.

Table 7: Monitor Test Result

| No. | Name | Age | Specimen | Behind the Ear |       |      |       | Armpit |       | Sole  |       |
|-----|------|-----|----------|----------------|-------|------|-------|--------|-------|-------|-------|
|     |      |     |          | Score          | Sweat | M.G. | Aging | Score  | Sweat | Score | Sweat |
| 1   | N.O. | 24  | PLACEBO  | 6.8            | 2.00  | 0.00 | 0.00  | 7.2    | 1.78  | 7.0   | 1.50  |
|     |      |     | SAMPLE   | 7.4            | 1.87  | 0.00 | 0.00  | 1.00   | 2.52  | 5.1   | 1.18  |
| 2   | Y.W. | 27  | PLACEBO  | 3.1            | 1.13  | 0.00 | 0.00  | 6.0    | 1.93  | 6.1   | 1.20  |
|     |      |     | SAMPLE   | 5.5            | 1.60  | 0.00 | 0.00  | 6.1    | 1.93  | 2.1   | 0.60  |
| 3   | T.T. | 24  | PLACEBO  | 6.5            | 2.07  | 0.00 | 0.00  | 16.1   | 2.93  | 6.3   | 1.33  |
|     |      |     | SAMPLE   | 5.2            | 1.60  | 0.00 | 0.00  | 7.5    | 2.20  | 6.4   | 1.40  |
| 4   | Y.I. | 23  | PLACEBO  | 8.0            | 2.07  | 0.00 | 0.00  | 11.7   | 2.33  | 4.4   | 1.20  |
|     |      |     | SAMPLE   | 8.1            | 2.13  | 0.00 | 0.00  | 9.9    | 2.13  | 9.0   | 1.67  |
| 5   | K.S. | 34  | PLACEBO  | 6.6            | 1.80  | 0.00 | 0.00  | 14.2   | 3.00  | 9.3   | 1.87  |
|     |      |     | SAMPLE   | 3.5            | 1.00  | 0.00 | 0.00  | 10.1   | 2.40  | 6.3   | 1.40  |
| 6   | N.K. | 38  | PLACEBO  | 5.1            | 1.53  | 0.00 | 0.00  | 10.7   | 2.07  | 5.4   | 1.27  |
|     |      |     | SAMPLE   | 7.1            | 1.80  | 0.00 | 0.00  | 11.9   | 2.33  | 9.6   | 1.93  |
| 7   | Y.Y. | 33  | PLACEBO  | 3.3            | 1.53  | 0.00 | 0.00  | 5.2    | 2.00  | 5.8   | 1.47  |
|     |      |     | SAMPLE   | 2.7            | 1.13  | 0.00 | 0.00  | 1.7    | 1.00  | 2.1   | 0.60  |
| 8   | A.M. | 30  | PLACEBO  | 4.7            | 1.80  | 0.00 | 0.00  | 9.5    | 2.53  | 8.1   | 1.87  |
|     |      |     | SAMPLE   | 5.2            | 1.73  | 0.00 | 0.00  | 11.7   | 2.67  | 8.1   | 1.80  |
| 9   | K.B. | 44  | PLACEBO  | 12.4           | 3.00  | 0.00 | 0.00  | 19.9   | 3.33  | 12.0  | 2.33  |
|     |      |     | SAMPLE   | 7.4            | 2.25  | 0.00 | 0.00  | 22.5   | 3.83  | 8.3   | 2.00  |
| 10  | M.O. | 48  | PLACEBO  | 10.8           | 1.83  | 0.00 | 0.00  | 11.0   | 2.67  | 5.0   | 1.00  |
|     |      |     | SAMPLE   | 8.3            | 1.92  | 0.00 | 0.00  | 9.8    | 2.42  | 6.6   | 1.25  |
| 11  | R.I. | 47  | PLACEBO  | 13.3           | 2.58  | 0.00 | 0.00  | 12.2   | 2.50  | 8.4   | 1.83  |
|     |      |     | SAMPLE   | 7.5            | 2.42  | 0.00 | 0.00  | 16.3   | 3.42  | 9.6   | 2.08  |
| 12  | K.H. | 42  | PLACEBO  | 8.8            | 2.00  | 0.00 | 0.00  | 18.7   | 3.17  | 6.0   | 1.08  |
|     |      |     | SAMPLE   | 5.1            | 1.75  | 0.00 | 0.00  | 15.3   | 3.08  | 6.1   | 1.33  |
| 13  | M.I. | 53  | PLACEBO  | 7.7            | 1.87  | 0.00 | 0.00  | 13.0   | 2.53  | 6.0   | 1.13  |
|     |      |     | SAMPLE   | 8.0            | 1.67  | 0.00 | 0.00  | 16.6   | 2.95  | 4.7   | 1.13  |
| 14  | T.H. | 51  | PLACEBO  | 4.2            | 1.47  | 0.00 | 0.00  | 6.1    | 2.00  | 4.5   | 1.00  |
|     |      |     | SAMPLE   | 1.7            | 0.87  | 0.00 | 0.00  | 4.5    | 1.47  | 2.7   | 0.60  |
| 15  | N.A. | 57  | PLACEBO  | 3.7            | 1.07  | 0.00 | 0.00  | 17.6   | 3.33  | 8.5   | 1.87  |
|     |      |     | SAMPLE   | 3.2            | 0.80  | 0.00 | 0.00  | 13.2   | 2.73  | 7.8   | 1.67  |
| 16  | M.H. | 50  | PLACEBO  | 7.3            | 2.07  | 0.00 | 0.00  | 18.1   | 3.13  | 9.0   | 1.73  |
|     |      |     | SAMPLE   | 6.0            | 1.60  | 0.00 | 0.00  | 13.1   | 2.73  | 9.8   | 1.87  |

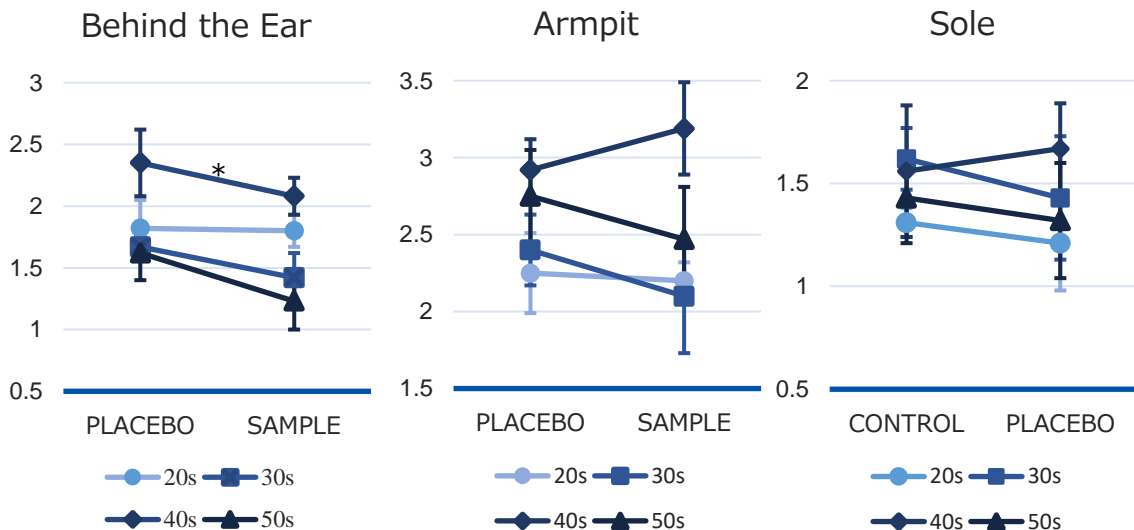
- Values in the table are average values of measurements performed 3 times a day in the morning, during the day, and at night for 5 days.
- The total point indicates the odor score out of 100 points. Points for Sweat, Middle Greasy, and Aging Odors indicate the intensity of the odor out of 10 points.



- The score of odor behind the ear slightly increased in the group of test subjects in their 20s when compared to the application of placebo. However, the odor tended to decrease among those in their 30s, 40s and 50s.
- Although the underarm odor slightly increased among those in their 40s when compared to the application of placebo, it decreased among those in their 20s, 30s and 50s.
- Odor of the sole was reduced more via the application of a lotion containing cosmeHerbest™ BLACK CUMIN than the placebo in all age groups.

Significantly different from control,  $**p < 0.01$

Fig. 11: Comparison of Body odor by site by age (Total Score)



- All values indicate sweat odor scores out of 10 points. Although the odor score for the armpit and sole increased among those in their 40s when compared to the application of the placebo, the odor intensity decreased overall in other age groups.

Significantly different from control,  $*p < 0.05$

Fig. 12: Comparison of Body odor by site by age (Sweat odor)

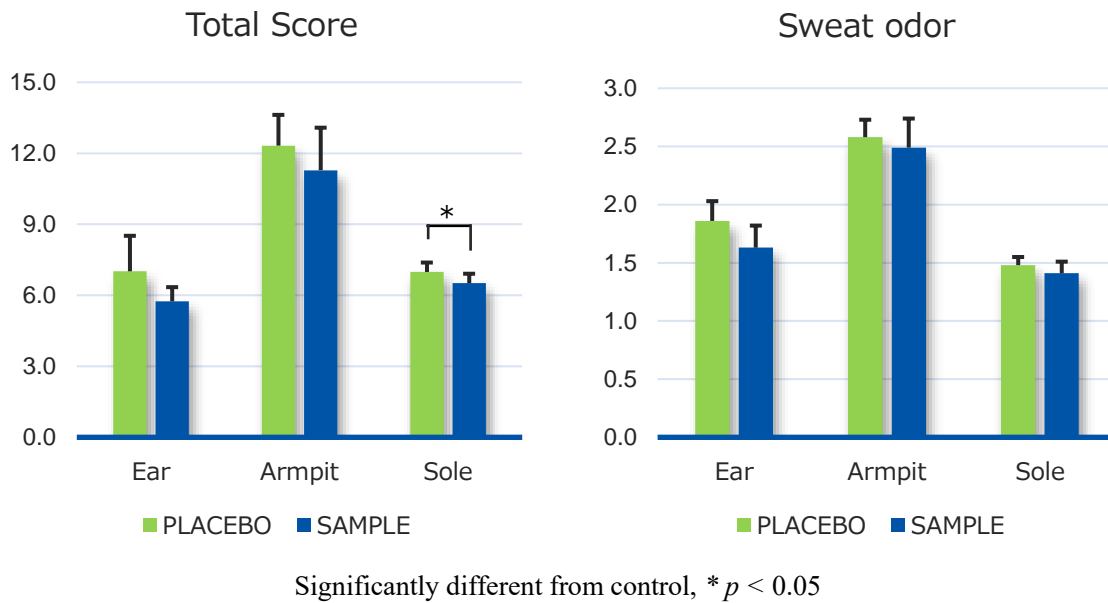


Fig.13: Comparison of Body odor by site (Whole age)

<Consideration>

The total odor score in all age groups and the average sweat odor in each part were calculated and compared between the application of lotions containing cosmeHerbest™ BLACK CUMIN and the placebo lotion. As a result, lotions containing cosmeHerbest™ BLACK CUMIN were confirmed to act to reduce odors. Middle-age greasy odor (diacetyl) or aging odor (Nonenal) was not detected behind the ear for the entire two-week (10 days) measurement period when the subjects applied the placebo or samples.

**Summary of the Result**

|                 | Sweat Odor<br>(Armpit, Sole) |   | Middle-age Greasy Odor<br>(Back of Head) | Aging Odor<br>(Trunk) |
|-----------------|------------------------------|---|--|-----------------------|
| Age             | Mid-10s to Mid-30s           |   | Mid-30s to Mid-50s                       | Mid-50s and Older     |
| Odor Components | - Isovaleric acid            | ◎ | - Diacetyl                               | △                     |
|                 | - <i>n</i> -Butyric acid     | ◎ |  |                       |
|                 | - Acetic acid                | ○ |  |                       |
|                 | - Ammonia                    | ◎ |  |                       |
|                 |                              |   |  | - 2-Nonenal           |
|                 |                              |   |  | ◎                     |

Deodorizing Rate: ◎: 50% or higher, ○: 25% or higher but below 50%,  
 △: 10% or higher but below 25%, ×: Below 10%

**As described above, cosmeHerbest™ BLACK CUMIN has been confirmed to have an effect to reduce body odors in all age groups.**

## 11. Stability Test

### 11-1 Long Term Stability

Store cosmeHerbest™ BLACK CUMIN as it was, in a cool dark place at 4° C, room temperature, window side and at 40° C, observed for 3 months, and determined optical density at 450nm and observe color of liquid, change of smell, and presence of precipitates.

<Test sample>

cosmeHerbest™ BLACK CUMIN, Lot No. N-802, undiluted

<Test Result>

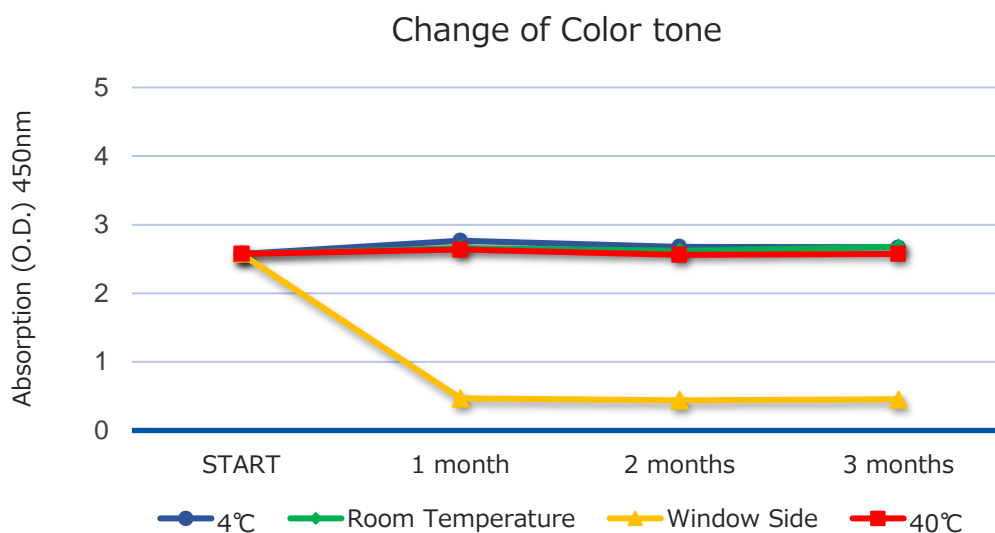


Fig.14: Long Term Stability Test (Color tone)

<Consideration>

Throughout the three months, there was a change in color in sunlight (window side). Also, the color tone was stable was stored in refrigerated (4 ° C), room temperature, 40 ° C. However, the viscosity increased in the refrigerator, crystals derived from plant extracts precipitated. Also, products stored at the window side and at 40 ° C are gradually stronger in oxidizing odor, and storage of the product at room temperature is recommended.



## 11-2 Long Term stability of Thymoquinone in the Product

We quantitatively analyzed the time course of Thymoquinone contained in cosmeHerbest™ BLACK CUMIN.

### <Test Sample>

Store cosmeHerbest™ BLACK CUMIN as it was, in a cool dark place at 4° C, room temperature, window side and at 40° C, observed for 3 months, and determined assay of Thymoquinone in cosmeHerbest™ BLACK CUMIN.

### <Test Result>

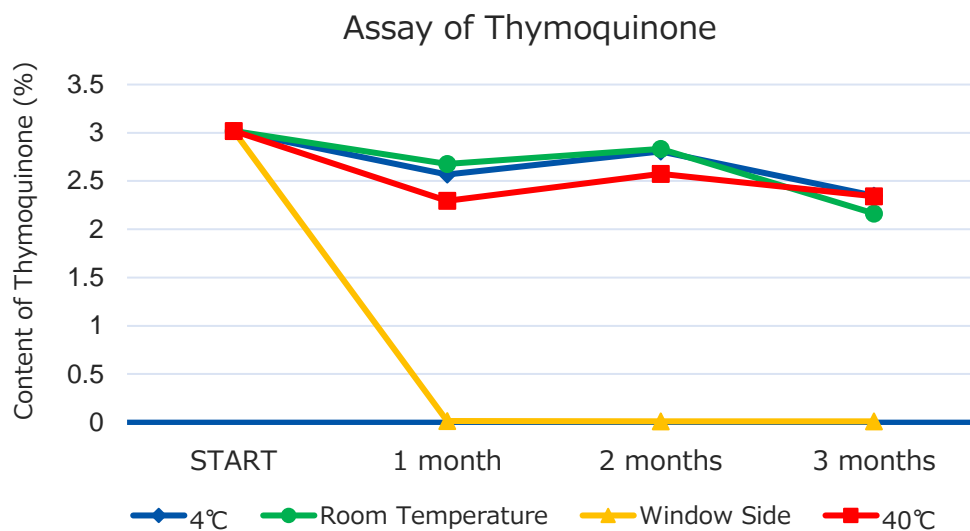


Fig.15: Long Term Stability of Thymoquinone (Assay)

### <Consideration>

As the results of the long-term stability test conducted in 11-1, it was found that the product stored at the window was strongly discolored, and the content of thymoquinone abruptly decreased accordingly. In addition, at 40 °C storage, there was no tendency for color fading and rapidly decline in thymoquinone content, but a tendency toward stronger oxidation odor was observed. Therefore, please keep it at 10 ~ 25 °C shading the light and use as soon as possible.

## 12. Compatibility (○ : Clear, △ : Turbid, × : Separation / Precipitation)

|                         | (%)  | Trade Name   | INCI Name                                  | Result |      |
|-------------------------|------|--|--|--------|------|
|                         |      | Manufacturer   |  | 1hr    | 24hr |
| <b>Cation</b>           | 3.0  | QUARTAMIN 86W<br>Kao Corporation                           | Steartrimonium Chloride / Water            | △      | ○    |
| <b>ANION</b>            | 10.0 | SOYPON SLE<br>Kawaken Fine Chemical Co., Ltd.              | Sodium Lauroyl Sarcosinate                 | △      | ×    |
|                         | 10.0 | EMAL 20C<br>Kao Corporation                                | Sodium Laureth Sulfate / Water             | △      | ×    |
|                         | 10.0 | AMISOFT CT-12S<br>Ajinomoto Co., Inc.                      | Water / TEA-Cocoyl Glutamate               | △      | ×    |
| <b>NONION</b>           | 10.0 | PYROTER GPI-25<br>Nihon Emulsion Co., Ltd.                 | Glycereth-25 PCA Isostearate               | △      | ×    |
|                         | 10.0 | SALACOS PG-218<br>Nisshin Oilio Group Co., Ltd.            | Polyglyceryl-10 Dioleate / Tocopherol      | △      | ×    |
|                         | 10.0 | RHEODOL 460V<br>Kao Corporation                            | Sorbeth-60 Tetraoleate                     | △      | ×    |
|                         | 10.0 | RHEODOL TW-0120V<br>Kao Corporation                        | Polysorbate 80                             | △      | ×    |
| <b>Ampho-<br/>teric</b> | 5.0  | AMPHITOL 20AB<br>Kao Corporation                           | Lauramidopropyl Betaine                    | △      | ×    |
|                         | 10.0 | SOFTAZOLINE LSB 29% aq.<br>Kawaken Fine Chemical Co., Ltd. | Lauramidopropyl Hydroxysulfate / Water     | △      | ×    |
| <b>SILICONE</b>         | 10.0 | KF-96A-10CS<br>Shin-Etsu Chemical Co., Ltd.                | Dimethicone                                | ×      | ×    |
|                         | 10.0 | KF-96A-300CS<br>Shin-Etsu Chemical Co., Ltd.               | Dimethicone                                | △      | ×    |
|                         | 10.0 | KF-995<br>Shin-Etsu Chemical Co., Ltd.                     | Cyclopentasiloxane                         | ○      | ○    |
|                         | 10.0 | Silwet L-7604<br>Momentive Performance Materials           | PEG-8 Dimethicone                          | △      | ×    |
|                         | 10.0 | Silwet L-7622<br>Momentive Performance Materials           | PEG-8 Dimethicone                          | ○      | ×    |
| <b>EMOLIENT·SOLVENT</b> | 1:1  | AMIER MA-HD<br>Nihon Emulsion Co., Ltd.                    | Hexyldecyl Myristoyl Methylaminopropionate | ○      | ○    |
|                         | 1:1  | EMALEX INTD-139<br>Nihon Emulsion Co., Ltd.                | Isotridecyl Isononanoate                   | ○      | ○    |
|                         | 1:1  | T.I.O<br>Nisshin Oilio Group Co., Ltd.                     | Triethylhexanoin                           | ○      | ○    |
|                         | 1:1  | MARCASOL R<br>Maruzen Petrochemical Co., Ltd.              | Isododecane                                | ○      | ○    |
|                         | 1:1  | PARAFOL 12-97<br>SASOL Ltd.                                | Dodecane                                   | ○      | ○    |
|                         | 1:1  | NIKKOL SUGAR SQUALANE<br>NIKKO Chemicals Co., Ltd.         | Squalane                                   | ○      | ○    |
| <b>POLYMER</b>          | 1.0  | Bio-HA 1% Solution (MP-PE) N<br>SHISEIDO Co., Ltd.         | Water / Sodium Hyaluronate / Methylparaben | ×      | ×    |

The concentration of cosmeHerbest™ BLACK CUMIN was adjusted to 1% with triethylhexanoin, the concentration of various cosmetic ingredients was adjusted to the values shown in the table with purified water, and the materials were mixed. Then, the condition of the mixture solution was observed one hour later and 24 hours later.

To test the compatibility of the product with emollient agents and solvents, the product was mixed at a 1:1 capacity ratio and the condition was checked.

### 13. Toxicological Safety Study

| Product Name                    |                   | cosmeHerbest™ BLACK CUMIN                                 |  |
|---------------------------------|-------------------|---|--|
| Safety Test Item                | Test Result       | Test Method   |  |
| Acute Oral Toxicity Test        | Not performed     |   |  |
| Primary Skin Irritation Test    | No Irritation     | LabCyte EPI method / OECD439<br>1% Conc. with Mineral oil |  |
| Accumulated Skin Irritancy Test | No Irritation     | RIPT method (50 people)<br>1% Conc. with Mineral oil      |  |
| Sensitization Test              | No Irritation     | RIPT method (50 people)<br>1% Conc. with Mineral oil      |  |
| Photo Toxicity Test             | Negative          | ROS Assay / OECD495<br>1% Conc. With DMSO                 |  |
| Photo Sensitization Test        | Not performed     |   |  |
| Eye Irritation Test             | No Irritation     | EpiOcular™ method / OECD492<br>1% Conc. with Mineral oil  |  |
| Mutagenicity Test               | Negative          | Ames method (TA98, TA100)<br>Undiluted                    |  |
| Human Patch Test                | (-)13, (±)1, (+)1 | 15 people<br>1% Conc. with White Vaseline                 |  |

## 14. Recommended Plans and Guide Formulation

Provided by Nihon Emulsion Co., Ltd.

- Deodorant Lotion
- Deodorant Cream
- Antiperspirant
- Body Lotion
- Foot-care Spray
- Paper-Towel
- Softener
- Dipper

### 14-1 Formulation Example 1 Deodorant Lotion / Modified DL-13

| No. | Trade Name                | Manufacturer             | %      | INCI Name  |
|-----|---------------------------|--------------------------|--------|--|
| 1   | AMITER MA-HD              | Nihon Emulsion Co., Ltd. | 0.50   | Hexyldecyl Myristoyl Methylaminopropionate   |
| 2   | EMALEX ML-158             | Nihon Emulsion Co., Ltd. | 2.00   | PEG-50 Hydrogenated Castor Oil Triisostearate, PEG-60 Hydrogenated Castor Oil, Ceteth-20 |
| 3   | EMALEX HC-30              | Nihon Emulsion Co., Ltd. | 1.00   | PEG-30Hydrogenated Caster Oil  |
| 4   | cosmeHerbest™ BLACK CUMIN | Oryza Oil & Fat Chemical | 0.50   | Nigella sativa Seed Extract, Rosmarinus Officinalis (Rosemary) Leaf Extract              |
| 5   | Allantoin                 |                          | 0.20   | Allantoin  |
| 6   | CAE                       | Ajinomoto Co., Inc.      | 0.20   | PCA Ethyl Cocoyl Arginate  |
| 7   | Propylene Glycol          |                          | 2.00   | Propylene Glycol   |
| 8   | PEG 4000                  |                          | 3.00   | PEG-75   |
| 9   | Glycerine                 |                          | 10.00  | Glycerin   |
| 10  | Water                     |                          | 10.00  | Water  |
| 11  | Natrosol 250HHR (1% sol.) | Ashland                  | 59.90  | Hydroxyethyl Cellulose, Water  |
| 12  | Methylparaben             |                          | 0.20   | Methylparaben  |
| 13  | Fragarance                |                          | 0.10   | Fragrance  |
| 14  | PURPLE TEA Extract-LC     | Oryza Oil & Fat Chemical | 0.50   | Water, Butylene Glycol, Camellia Sinensis Leaf Extract                                   |
| 15  | Ethanol (95%)             |                          | 10.00  | Alcohol  |
|     |                           |                          | 100.00 |  |

#### Preparation Method

- 1) Mix and dissolve Ingredients No. 1 to 4 at 70°C.
- 2) Mix and dissolve Ingredients No. 5 to 11 at 75°C.
- 3) While stirring 1) B by homogenizer, add 2) and mix well and cool at 40°C.
- 4) Mix and dissolve Ingredients No. 12 to 15 at room temperature, add into 3) and cool as the product.

Note: Thoroughly study and examine the temporal stability, safety including skin irritation, and restrictions such as the patent law for this formulation before use.

## 14-2 Formulation Example 2 Deodorant Cream / Modified IDC-16

| No. | Trade Name                | Manufacturer             | %      | INCI Name  |
|-----|---------------------------|--------------------------|--------|--|
| 1   | EMALEX DISG-2EX           | Nihon Emulsion Co., Ltd. | 3.00   | Polyglyceryl-2 Diisostearate   |
| 2   | Butylparaben              |                          | 0.10   | Butylparaben   |
| 3   | l-Menthol                 |                          | 0.15   | Menthol  |
| 4   | dl-Camphor                |                          | 0.05   | Camphor  |
| 5   | KF-995                    | Shin-Etsu Chemical       | 4.50   | Cyclopentasiloxane   |
| 6   | Quaternium-18 Hectorite   |                          | 1.00   | Quaternium-18 Hectorite  |
| 7   | EMALEX SS-5051            | Nihon Emulsion Co., Ltd. | 1.00   | PEG-10 Dimethicone / Dimethicone   |
| 8   | AMITER MA-HD              | Nihon Emulsion Co., Ltd. | 5.00   | Hexyldecyl Myristoyl Methylaminopropionate                                     |
| 9   | Squalane                  |                          | 3.00   | Squalane   |
| 10  | cosmeHerbest™ BLACK CUMIN | Oryza Oil & Fat Chemical | 1.00   | Nigella sativa Seed Extract,<br>Rosmarinus Officinalis (Rosemary) Leaf Extract |
| 11  | Phenoxyethanol            |                          | 0.10   | Phenoxyethanol   |
| 12  | ORYZATOCOTRIENOL-90       | Oryza Oil & Fat Chemical | 0.10   | Tocotrienols, Tocopherol,<br>Oryza Sativa (Rice) Bran Oil                      |
| 13  | TCG-M                     | Kokyu Alcohol Industry   | 6.00   | Caprylic/Capric Triglyceride   |
| 14  | cosmeHerbest™ KIWI        | Oryza Oil & Fat Chemical | 0.60   | Water, Propanediol,<br>Actinidia Chinensis Seed Extract                        |
| 15  | Aluminum Chlorohydrate    |                          | 2.00   | Aluminum Chlorohydrate   |
| 16  | Water                     |                          | 62.40  | Water  |
| 17  | Glycerin                  |                          | 5.00   | Glycerin   |
| 18  | Sorbitol Sol. (70%AI)     |                          | 5.00   | Water, Sorbitol  |
|     |                           |                          | 100.00 |  |

## Preparation Method

- 1) Mix and dissolve Ingredients No. 1 to 4 at 55°C. Further, add Ingredients No. 5 to 13, mix well and disperse.
- 2) Mix and dissolve Ingredients No. 14 to 18.
- 3) While stirring 1) to add 2) little by little to emulsify as the product.

Note: Thoroughly study and examine the temporal stability, safety including skin irritation, and restrictions such as the patent law for this formulation before use.

### 14-3 Formulation Example 3 Antiperspirant / Modified SSFM-59A

| No. | Trade Name                | Manufacturer                | %      | INCI Name  |
|-----|---------------------------|-----------------------------|--------|--|
| 1   | AMITER LG-1600            | Nihon Emulsion Co., Ltd.    | 0.35   | Dihexyldecyl Lauroyl Glutamate   |
| 2   | EMALEX 102                | Nihon Emulsion Co., Ltd.    | 0.95   | Ceteth-2   |
| 3   | EMALEX 620                | Nihon Emulsion Co., Ltd.    | 0.50   | Steareth-20  |
| 4   | cosmeHerbest™ BLACK CUMIN | Oryza Oil & Fat Chemical    | 0.20   | Nigella sativa Seed Extract,<br>Rosmarinus Officinalis (Rosemary) Leaf Extract |
| 5   | Aluminum Chlorohydrate    |                             | 20.00  | Aluminum Chlorohydrate   |
| 6   | Water                     |                             | 58.00  | Water  |
| 7   | CAE                       | Ajinomoto Co., Inc.         | 2.00   | PCA Ethyl Cocoyl Arginate  |
| 8   | Methylparaben             |                             | 0.20   | Methylparaben  |
| 9   | Genamin KDM-P             | Clariant Specialty Products | 10.00  | Behentrimonium Chloride  |
| 10  | Water                     |                             | 18.90  | Water  |
|     |                           |                             | 100.00 |  |

#### Preparation Method

- 1) Mix and dissolve Ingredients No. 1 to 4 at 60°C. (Phase A)
- 2) Mix and dissolve Ingredients No. 5 and 6 at 75°C. (Phase B)
- 3) Mix and dissolve Ingredients No. 7 to 10 at 75°C. (Phase C)
- 4) Add Phase C into Phase B, mix by paddle, further, adding Phase A to emulsifier for 3 minutes
- 5) Keep cool until 30°C as the product

Note: Thoroughly study and examine the temporal stability, safety including skin irritation, and restrictions such as the patent law for this formulation before use.

#### 14-4 Formulation Example 4 Body Lotion / Modified FDA-06

| No. | Trade Name                            | Manufacturer                        | %      | INCI Name  |
|-----|---------------------------------------|-------------------------------------|--------|--|
| 1   | ELDEW PS-203                          | Ajinomoto Co., Inc.                 | 1.50   | Phytosteryl/Octyldodecyl Lauroyl Glutamate                                     |
| 2   | <b>EMALEX INTD-139</b>                | <b>Nihon Emulsion Co., Ltd.</b>     | 1.50   | Isotridecyl Isononanoate   |
| 3   | <b>cosmeHerbest™ BLACK CUMIN</b>      | <b>Oryza Oil &amp; Fat Chemical</b> | 1.00   | Nigella Sativa Seed Extract,<br>Rosmarinus Officinalis (Rosemary) Leaf Extract |
| 4   | Purified JOJOBA Oil                   | Yokozeki Oil & Fat Ind.             | 2.00   | Simmondsia Chinensis (Jojoba) Seed Oil   |
| 5   | KF-96A (6 c/s)                        | Shin-Etsu Chemical                  | 1.00   | Dimethicone  |
| 6   | Cetanol H                             | Kokyu Alcohol Kogyo                 | 3.00   | Cetearyl Alcohol   |
| 7   | <b>EMALEX EGS-A</b>                   | <b>Nihon Emulsion Co., Ltd.</b>     | 1.00   | Glycol Stearate  |
| 8   | <b>EMALEX GMS-F</b>                   | <b>Nihon Emulsion Co., Ltd.</b>     | 1.00   | Glyceryl Stearate  |
| 9   | <b>EMALEX 8100</b>                    | <b>Nihon Emulsion Co., Ltd.</b>     | 1.00   | PEG-100 Stearate   |
| 10  | <b>ORYZATOCOTRIENOL™ -90</b>          | <b>Oryza Oil &amp; Fat Chemical</b> | 0.05   | Tocotrienol, Tocopherol,<br>Oryza Sativa (Rice) Bran Oil                       |
| 11  | Phenoxyethanol                        |                                     | 1.00   | Phenoxyethanol   |
| 12  | EDTA-2Na (1% soln.)                   |                                     | 1.00   | Water, Disodium EDTA   |
| 13  | Glycerine                             |                                     | 10.00  | Glycerine  |
| 14  | KeltrolCG-T* <sup>5</sup> (1% soln.)  | CP Kelco                            | 10.00  | Water, Xanthan Gum   |
| 15  | Carbopol 940* <sup>6</sup> (1% soln.) |                                     | 10.00  | Water, Carbomer  |
| 16  | <b>cosmeHerbest™ YUZU</b>             | <b>Oryza Oil &amp; Fat Chemical</b> | 1.00   | Water, Propanediol, Citrus Junos Seed Extract                                  |
| 17  | <b>cosmeHerbest™ ORANGE</b>           | <b>Oryza Oil &amp; Fat Chemical</b> | 1.00   | Water, Butylene Glycol, Citrus Unshiu Peel Extract                             |
| 18  | Water                                 |                                     | 50.45  | Water  |
| 19  | Potassium Hydroxide (10% soln.)       |                                     | 2.50   | Water, Potassium Hydroxide   |
|     |                                       |                                     | 100.00 |  |

#### Preparation Method

- 1) Mix and dissolve Ingredients No. 1 to 11 at 80°C. (Phase A)
- 2) Mix and dissolve Ingredients No. 12 to 18 at 75°C. (Phase B)
- 3) While stirring Phase B by homogenizer, add Phase A, further, add Ingredients No.19 and mix well. (3000rpm, 3 minutes)
- 4) Then, mix by Paddle and cool at 40°C as the product.

Note: Thoroughly study and examine the temporal stability, safety including skin irritation, and restrictions such as the patent law for this formulation before use.



## 15. Product Specification

| Item   | Specification  | Remarks                                   |
|--|--|---|
| Product Name                                   | cosmeHerbest™ BLACK CUMIN  |   |
| Appearance                                     |  |   |
| • Color  | Brown to slight yellowish brown oily liquid  |   |
| • Odor   | Characteristic odor  |   |
| Identification                                 |  |   |
| 1) IR Spectrum                                 | It exhibits wavelength around 2926cm <sup>-1</sup> 、1745cm <sup>-1</sup> 、1458cm <sup>-1</sup> . |   |
| 2) Thymoquinone                                | Positive   | TLC<br>Rf = around 0.8                    |
| Specific Gravity d <sub>20</sub> <sup>20</sup> | 0.915 to 0.925   | Method 1, C                               |
| Refractive Index n <sub>D</sub> <sup>20</sup>  | 1.472 to 1.478   |   |
| Purity Test                                    |  |   |
| 1) Heavy Metals                                | 10 ppm max.  |   |
| 2) Arsenic                                     | 1 ppm max.   |   |
| Residue on Ignition                            | 0.5% max.  | 1g, 550°C, Method 2<br>25mL,              |
| Moisture                                       | 1.0% max.  | Volumetric titration,<br>Direct titration |
| Assay  |  |   |
| • Thymoquinone                                 | 1.5 to 4.5 %   |   |
| Microbial Test                                 |  |   |
| 1) Bacterial count                             | 1 × 10 <sup>2</sup> /g max.  | Hygiene Test Method                       |
| 2) Yeast, Mold                                 | 1 × 10 <sup>2</sup> /g max.  | Hygiene Test Method                       |
| 3) Coliform                                    | Negative   | Hygiene Test Method                       |

These standards and test method are referred to General Notices and General Tests, Processes and Apparatus of The Japanese Standards of Quasi-drug Ingredients, unless otherwise specified.

## 16. Cosmetic Regulation and Certification

|                                  |   |  |
|----------------------------------|---|--|
| 16-1 Japanese Labelling Name     | : | ニゲラサチバ種子エキス<br>ローズマリー葉エキス  |
| 16-2 Japanese Quasi-drug Name    | : | None   |
| 16-3 INCI Name                   | : | Nigella Sativa Seed Extract<br>Rosmarinus Officinalis (Rosemary) Leaf Extract          |
| 16-4 已使用化粧品原料名称目录<br>(IECIC2021) | : | 栽培黑种草 (Nigella Sativa) 籽提取物<br>迷迭香 (Rosmarinus Officinalis) 叶提取物                       |
| 16-7 ISO16128                    | : | Natural Index 1<br>Natural Origin Index 1<br>Organic Index 1<br>Organic Origin Index 1 |

## 17. Others

- 17-1 Packaging style  
Outer: Carton box, Inner: 1 kg tin-can
- 17-2 Storage Condition and Shelf Life  
2 years from the manufacturing date  
Store it in a cool, dry, ventilated area with desiccant. Keep it away from high temperature and sunlight, and store it in a closed container.

## 18. Reference

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- 2) General Odor Research on Male Bodily Odors, Mandom Corporation Homepage  
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- 9) JP2014-73976A
- 10) H. Khither et al. / ARRB, 25(5): 1-9, 2018; Article no. ARRB.40165
- 11) <https://kunkunbody.konicaminolta.jp/>

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Factory in Ichinomiya

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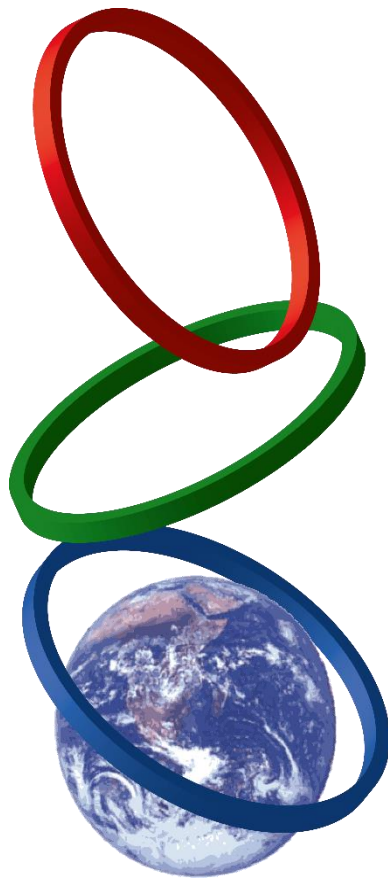
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